MARCH 2019 # 74

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Image of the Month

Terror on the High Seas

Copepods (pictured) imprint seawater with a unique chemical signature that induces defensive traits in the phytoplankton they eat, according to a Swedish study. The University of Gothenburg researchers used LC-MS to measure chemicals released by the copepods (copepodamides) and found that, as the levels of copepodamides increased, the phytoplankton generated more toxins and formed smaller colonies – tactics designed to evade predation.

Reference: E Selander et al., "Copepods drive large-scale trait-mediated effects in marine plankton", Sci Adv, 5, eaat5096 (2019). DOI: 10.1126/sciadv.aat5096.

Credit: Erik Selander.

Would you like your photo featured in Image of the Month? Send it to charlotte.barker@texerepublishing.com

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A tribute to the artwork for Kraftwerk's classic 1978 album, The Man Machine. Robot image courtesy of Mike MacKenzie via www.vpnsrus.com

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On the Up and Up

The cannabis industry is moving into the mainstream – and the big analytical players are on board.

I recently returned from San Diego, California, where I represented our sister publication – The Cannabis Scientist (1) – at a two-day event exploring all scientific aspects of the (in)famous plant. On my way to the Emerald Conference, I chatted to various interesting characters – much like Dorothy (though my yellow brick road felt much longer – and there were no lions). Inevitably, the question would arise: "So, what takes you to California?" When I replied that I was attending a cannabis conference, I was surprised (and perhaps a little disappointed) that I didn't raise a single eyebrow. Instead, responses ranged from polite interest to outright enthusiasm.

This positive response from strangers reminded me just how much public perception of cannabis has shifted in recent years – and the conference itself showed me how the industry has changed likewise. Even in the three short years since we launched The Cannabis Scientist, the difference is palpable. Back in 2016, a number of instrument manufacturers kept a low profile in an area that was still considered "edgy" at best. This year at Emerald ("the most technical cannabis science conference in the industry," as the organizers proudly say), there was no such reticence; five of the top sponsorship spots were taken by major international equipment and consumable companies – all of whom are releasing masses of application notes, technical information and educational materials specific to the cannabis market.

A growing number of scientific conferences and journals are springing up and, though there are still those who believe cannabis cures everything from cancer to the common cold, the deluge of "anecdata" is gradually being replaced by scientifically valid clinical trials and large-scale observational studies, which regularly appear in the world's biggest scientific publications.

In the early days of legalization, the cannabis industry was often referred to as the Wild West – complete with a "green rush" of amateur growers, dispensaries and testing labs. Now, as regulations become stricter and as scientific understanding increases, the industry is becoming more pioneer than cowboy.

From the start, The Cannabis Scientist has maintained a strong scientific focus – just like all of our other publications – so it was particularly gratifying to hear scientists at the Emerald Conference saying that they value the magazine's intelligent, nuanced approach. The USP of our magazines has always been to take a high-level view of complex concepts in an accessible way, without either dumbing down or getting lost in technical details – and we believe that is especially valuable in a field undergoing rapid change.

Charlotte Barker
Editor

Reference

1. *https://theanalyticalscientist.com/thecannabisscientist*

Upfront

Reporting on research, personalities, policies and partnerships that are shaping analytical science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker@texerepublishing.com

Biomarkers Beyond Our Wildest Dreams

Could glycosylation patterns associated with a sleep behavior disorder predict the onset of neurodegenerative disease?

For someone with idiopathic REM sleep behavior disorder (iRBD), dreams can come true – literally. A loss of muscle paralysis during rapid eye movement (REM) sleep can cause patients with the disorder to physically act out their dreams. There is a pressing need to identify biomarkers that could help diagnose this condition – not just because the disorder itself can affect overall health, but also because iRBD is now considered to be an early indicator of an α-synucleinopathy related to various neurodegenerative diseases, such as Parkinson's. Now, research that focuses on glycosylation patterns has identified certain N-glycan structures that could serve as diagnostic biomarkers for patients with iRBD (1).

Although the quest for iRBD biomarkers has recently been driven by proteomics, a growing body of evidence has shown that glycosylation plays an important role in dynamic disease mechanisms and the onset of various pathological conditions. This led the researchers to compare the glycosylation patterns of nine iRBD patients with those of 10 healthy controls using liquid chromatography and mass spectrometry (LC-MS). The analysis of

permethylated glycans from blood sera found 59 N-glycan structures in the healthy cohort and 56 in the iRBD cohort. Sixteen of these N-glycan structures were significantly altered in the patients with iRBD, including the $HexNAc_6Hex_6$ species, which was only present in this group.

In addition, seven N-glycan isomers differed significantly between the two cohorts. Of these, the greatest differences were found in levels of $HexNAc_4$ Hex_5 Fuc_1 $NeuAc_1$ and $HexNAc_4$ Hex_5 Fuc_1 $NeuAc_2$, which were higher in the iRBD group. By acting as diagnostic biomarkers for iRBD, these findings could provide an important window into the underlying neurodegenerative diseases such patients may possess.

To achieve accurate separation, identification, and quantification of isomeric glycans, the researchers needed to address issues with the ability of LC-MS to separate isomers. "Our group has adopted a sophisticated and advanced approach to optimize isomeric separation by using porous graphitized carbon stationary phase under a high temperature of 75°C," the authors explained in their study. The approach facilitated the "reliable identification and characterization of glycan structures, as well as precise and accurate quantitation of isomeric structures."

They emphasize that further studies with larger sample sizes are needed to confirm the reproducibility of their findings. Eventually, though, their discovery that a link could exist between glycosylation patterns and the presence of iRBD could be crucial for identifying patients at increased risk of neurodegenerative disorders. Although the potential for such predictions raises ethical questions around selecting patients to be tested – and mitigating the possible psychological effects of the results – the potential for developing molecular therapeutic targets to combat neurodegeneration is nevertheless enticing.

Reference

1. *X Dong et al., "LC-MS/MS glycomics of idiopathic rapid eye movement sleep behavior disorder", Electrophoresis, 39, 3096–3103 (2018). PMID: 30168606.*

Jumping on the NMR Pulse Train

A new polarization transfer technique promises improved sensitivity and rapid analysis times

Nobody has the time or inclination to wait over a century for nuclear magnetic resonance (NMR) results. In the past, such a delay would understandably have hampered analysis of complex structures – but now, Kong Ooi Tan, a postdoctoral researcher at the Massachusetts Institute of Technology (MIT), and his colleagues have developed a novel way of improving the sensitivity of NMR spectroscopy to massively reduce the time needed to study the structures of intricate molecules (1). Using the new approach, scientists should be able to analyze complicated molecular structures, such as that of the amyloid beta (Aβ) protein linked to Alzheimer's disease, in just one day – a fantastic improvement over the 110 years previous techniques would have required.

The polarization of an atom affects the sensitivity of NMR and can be increased to enhance sensitivity – for example, by using a stronger magnetic field. Dynamic nuclear polarization (DNP), an alternative technique developed by co-author Robert Griffin and colleague Richard Temkin (Associate Director of the Plasma Science and Fusion Center, MIT) over the past 25 years, boosts the polarization of NMR-active nuclei by transferring it from unpaired electrons of free radicals in the sample undergoing analysis. Traditional DNP can make NMR 100-fold more sensitive by continuously irradiating samples with high-frequency microwaves using a gyrotron. Unfortunately, it's a power-hungry method that falls down at higher magnetic field strengths.

Using magic-angle spinning (MAS) NMR recoupling sequences as inspiration, Tan's team decided to apply a train of microwave pulses separated by delays instead of continuously blasting the sample with microwaves. Carefully selecting the pulse length, power, and delay enhanced the polarization by a factor of up to 200 – similar to "traditional" DNP's capabilities, but requiring only 7 percent of the power. Moreover, the new technique can be used at higher magnetic field strengths, offering further sensitivity enhancements. In their paper, the researchers emphasized that the efficiency of their sequence "is primarily determined by the timing of the pulses and delays rather than the microwave power." Crucially, the timing can be controlled more reliably than the power, which can vary across a sample.

At the moment, the new technique has only been applied to standard test molecules in DNP juice (a glycerol/water mixture), but there are plans to study a range of low-abundance biological proteins, including Aβ, ion channels, and rhodopsin.

Reference

1. *KO Tan et al., "Time-optimized pulsed dynamic nuclear polarization", Sci Adv, 5, eaav6909 (2019).*

Caught in the Net

Which insecticide-infused mosquito nets are still effective? Mass spec has the answer

Insecticide-infused netting plays a vital role in limiting the spread of mosquito-borne diseases, but determining when a net has lost its potency poses a tricky technical challenge. Collaboration between pathologists and analytical scientists in the US has led to a potential solution – a mass spectrometric method to differentiate between effective and ineffective netting (1).

"We've developed a way to measure two of the most common insecticides used on any type of netting," said Fred Stevie, senior researcher at North Carolina State University's Analytical Instrumentation Facility, in a press release (2). Targeting permethrin, one of the most widely used insecticides in mosquito netting, the team used mass spectrometry to obtain chemical fingerprints of both the insecticide and the netting material. Using time-of-flight secondary ion mass spectrometry (ToF-SIMS) – which is based on the pattern of ions ejected from a sample's surface following bombardment with bismuth ions – the researchers were able to determine the overall makeup of the sample.

To validate their approach, the researchers gathered a variety of nets that had seen varying degrees of use, as well as real-world data on their efficacy. ToF-SIMS allowed the team to deduce the level of permethrin at which the nets became ineffective. For Stevie, the work could have global ramifications; "There are more than a billion nets out there, and our new technique can tell us how long the pesticide on those nets last," he said. "Ultimately, the technique could help us examine a range of fabrics embedded with insecticides, from military uniforms to high-end hiking gear."

References

1. *SC Smith et al., "Imaging and quantitative analysis of insecticide in mosquito net fibers using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)," PLoS One, 13, e0209119 (2018). PMID: 30586430.*
2. *M Shipman, "For first time, researchers can measure insecticide on surface of mosquito nets" (2019). Available at: https://bit.ly/2Fj8yy6. Accessed March 1, 2019.*

An Analytical Pill

Measuring gaseous biomarkers to help diagnose and monitor gut disorders

Credit: Atmo Biosciences.

Digestive disorders are common, and their symptoms are often incontrovertible – certainly to the tens of millions of patients worldwide who suffer from them. Diagnosis, however, is rarely so clear, which is why nearly one-third of all patients with gut disorders are unable to put a name to their condition. But a new device – an electronic capsule that can measure gaseous biomarkers and transmit the information wirelessly to waiting physicians (1) – may improve upon the accuracy of existing tests.

Kouroush Kalantar-zadeh, co-inventor of the capsule and Lead Scientific Advisor for the company set to commercialize it, explains: "In most cases, unless there is a visual marker like a wound or inflammation, prevention, diagnosis, and monitoring tool options are very limited for gut disorders."

It was an encounter with a gastroenterologist that inspired the new device; Kalantar-zadeh was initially asked to make existing breath tests more accurate, but concluded that the indirect measurement made the task impossible. Instead, he decided to create a capsule that patients can swallow – one that can directly sense and measure gases such as hydrogen, oxygen, and carbon dioxide – within the digestive system.

"The capsule, designed by engineer Nam Ha, moves along the gut after the ingestion and leaves the body with the natural motility of gastrointestinal tract – generally within 24 to 48 hours," Kalantar-zadeh explains. The capsule transmits data every five minutes to a handheld monitor the patient carries, which in turn sends the information via Bluetooth to a mobile phone for online monitoring or cloud storage. "So far, we have shown accuracy of near 100 percent in measuring hydrogen as a biomarker. In comparison, breath test accuracy is significantly lower." Kalantar-zadeh emphasizes that accuracy of gas measurements directly translates to accuracy in the prevention, diagnosis, and monitoring of gut disorders from malabsorption to inflammatory bowel disease. Soon, he hopes, the device will enter mass production and begin improving the lives of digestive disorder patients everywhere.

Reference

1. *RMIT University, "Diagnostic advance: gas-sensing capsule set to hit market by 2022" (2018). Available at: https://bit.ly/2ChCcCE. Accessed January 2, 2019.*

Managing Malaria with Smarter Sampling

How targeting subclinical populations with a rapid saliva test may aid in the quest to eradicate the disease

Efforts to eradicate certain infectious diseases, such as smallpox and polio, have generally proven fruitful – but one devastating infection remains stubborn in the face of multiple attempts: malaria. Now, researchers believe a simple saliva test that detects the infectious malarial parasite in asymptomatic carriers may provide a new way of looking at disease prevention (1).

Many efforts to tackle malaria have focused on targeting established disease. Rhoel Dinglasan, Associate Professor of Infectious Diseases at the University of Florida Emerging Pathogens Institute and the College of Veterinary Medicine, and also the senior author of the recent paper in Science Translational Medicine, shares the rationale behind his team's alternative approach: "Malaria can be looked at like an iceberg – we can treat the people at the tip of the iceberg with evident disease. But there are many people under the waterline that are contributing to the continuation of the disease, regardless of all the control efforts focused on malaria."

To generate a candidate biomarker list, the researchers performed a cross-sectional, multi-omics study of saliva from 364 children with subclinical infection. Using advanced mass spectrometry-based workflows, the researchers identified 60 proteins that appeared to be secreted into the saliva by the parasite. One in particular – PSSP17 – stood out as a potential biomarker, chiefly because of its relative abundance in all the samples. "We made antibodies to this marker – followed by the development of a simple test in our lab," says Dinglasan. Perhaps more promisingly, the test worked on both fresh and archived samples. "It allows us to analyze a large number of samples after they've been collected," says Dinglasan, who is hopeful that widespread testing of rural populations in Africa could, therefore, be conducted with relative ease.

The real litmus test for the tool was if it could successfully be translated to the clinic – or in this case, the local village. "We set up calls with multiple stakeholders – asking what they would want to see in a diagnostic test. Every time an idea that challenged us was put on the table, we went ahead in incorporating it into the design," says Dinglasan.

With the new and improved "saliva-based point-of-need (PON) lateral flow immunoassay test" in the bag, Dinglasan and his team went in pursuit of commercial backing. Enter ERADA Technology Alliance – a start-up biotech company located in Musina, South Africa. In a twist of fate, ERADA Managing Director, Benji Pretorius, had more than a business interest in the new test; in 2017, he survived a bout of the disease himself. "I was convinced that, if we could do better at detecting the signs and symptoms, we could save a great deal of sickness and suffering," he says. ERADA, with the support of its collaborators, is now putting in place the necessary steps to commercialize the technology. Pretorius is optimistic that the test will be available soon: "We are now in a good place to finalize the saliva-based test – and we plan to launch this much needed diagnostic tool in Africa in the next few years."

What about other infectious diseases? "We're focusing on malaria," says Pretorius. "But our technology, our drive, our vision – they aren't limited to malaria; we can apply many of our strengths to drive progress in other diagnostic areas."

References:

1. *D Tao, et al., Sci. Trans. Med. 11, 473 Epub ahead of print (2019).*
2. *ERADA Technology Alliance, (2019). Available at: http://www.eradatechnology.com Accessed January 14, 2014.*

In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.

Contact the editors at charlotte.barker @texerepublishing.com

It's Complicated

The potential market for complex generics is substantial, but navigating the FDA's guidance for proving "sameness" is a real minefield...

By Bérangère Tissot, General Manager at SGS Life Sciences.

Generic medicines are now an established part of the pharmaceutical supply chain and offer significant savings to health services, insurers and patients alike. More recently, biosimilars have entered the fray, and while the savings are not as great as with small molecule generics, they still help cut the costs of medicine. However, there is also a third category of product that falls between the two – the complex generic. These are products that may include complex: active ingredients, formulation, route of delivery, or even a mixture of ingredients.

The key to creating a new generic or biosimilar medicine and gaining regulatory approval is proving that it is safe and comparable to the originator product. For a small molecule generic, proving "sameness" between the two is relatively straightforward, relying heavily on a sub-set of well-defined analytical methods. Biologics are very different, because the exact nature of the product depends on how it is manufactured, leading regulators to demand clinical studies that prove the biosimilar is functionally comparable to the reference product.

Many of the complex generics currently being developed are peptides – albeit less than 40 amino acids long – and proving sameness for the active ingredients can be tricky. The same often applies to other molecule types that can be considered "complex" such as polyamino acids (for example, Copaxone (glatiramer acetate), which is a random combination of four amino acids) carbohydrates (which can also be sulfated as Enoxaparin or pentosan polysulfate), and naturally derived mixtures, such as estrogens.

European regulators tend to consider some of these complex generics products to be more like biologics (such as Enoxaparin), thus requiring clinical work. But the story is different in the US, where regulators are instead looking for proof that the molecules are the same, in the same way as a small molecule generic. While draft guidances were recently published for Enoxaparin and glatiramer acetate, they only provide the general areas where sameness needs to be demonstrated – and no details on how to actually demonstrate it. There's also limited technical direction – certainly not to the same level as a general chapter in the US Pharmacopeia – the guidance simply says that equivalence must be proved. In some respect, this is in agreement with a lot of guidances from regulatory authorities. However, for biological products, other documents such as the ICH Q6B guidelines do offer a list of critical parameters and possible techniques to be applied when characterizing a protein. In the case of complex generics, there is very little documentation to be used and, when it does exist, caution needs to be exercised on how to put the information provided into use. For example, the guidance for Enoxaparin refers to complex documents such as a petition that spans over almost a decade, which discusses what might be required and refers to about 133 publications that readers will want to check. Much of this might be obsolete, having been superseded by more recent and applicable research.

For complex generic peptide APIs, the FDA specifies that physicochemical properties, primary sequence, secondary structure, oligomer structure, and biological activities must all be assessed. While many complex APIs may be comprised of chains

of amino acids, they aren't proteins, so the typical protein toolbox isn't readily applicable. In fact, some are heterogeneous mixtures that may or may not have specific signatures or modifications, such as glatiramer acetate – for which there are no off-the-shelf tools at all.

For primary sequence or impurity characterization, there is a widespread, and mistaken, belief that mass spectrometry analysis will suffice, but this is rarely the case. If a peptide includes an unnatural amino acid that is an enantiomer of the naturally occurring version, mass spectrometry cannot unequivocally identify this because their mass will be the same. A technique such as chiral chromatography will be required in conjunction with sequence analysis if sameness is to be proved.

For these peptides, determining secondary and higher-order structures is quite complex. The techniques applicable to proteins simply aren't appropriate for smaller peptides, and the list of techniques suitable for this class of compounds is decidedly limited. How can these be used to create a comprehensive analytical strategy to prove sameness? To complicate matters even more, the FDA is clear that orthogonality in the definition of each quality attribute is recommended.

My take on this would be that the solution must use a lot of experimentally-driven evidences and an appropriate analytical strategy. The costs and timelines associated with this work are significant – and it would be easy for generics companies to embark on developing a complex generic, without fully realizing how much more challenging the process is, compared with a traditional small molecule. Even with a good analytical strategy at hand, there is the challenge of comparing it to the reference listed drug. Some of these peptides are formulated at extremely low concentrations – often less than a milligram per milliliter, and even down to the micrograms level. Vasopressin, for example, is typically formulated at approximately 37µg/ml, and calcitonin at 33µg/ml. Biophysical techniques to determine secondary structures are not applicable at such low concentrations and for such short chains. The formulation of the reference product also poses problems. Not only are they usually of low concentration, they are formulated with the inclusion of bacteriostatic ingredients, which are ultraviolet (UV) absorbents. Most secondary structure analysis techniques are based on UV methods, meaning these cannot be used on the formulated product.

New methods will have to be brought to the FDA that will work. But for the analytical scientist, this isn't as simple as finding the best method and running with it; it must also be demonstrated that the other methods won't work.

In my view, the key for all analytical sameness studies is in the preparation, planning and understanding of the technical and scientific challenges each complex generic API presents. Only if these are properly evaluated and defined in advance can any analytical package have a chance of being favorably looked upon. With the right planning, companies will be able to purchase enough reference listed drug material for all phases of the study, design fit-for-purpose studies for each of the quality attributes to be followed, and perform the experimentally-defined selection demonstrated-to-be-fit methods. Only then will this ultimately lead to straightforward analytical comparability studies.

Cannabis Analysis: Investing in Testing

Rigorous analysis is vital to keep cannabis consumers safe – and help drive forward medical advances.

By Cindy Orser, Chief Science Officer, DigiPath Labs, Las Vegas, Nevada, USA.

Over the course of my career, I've worked in microbiology, genetics, biochemistry, biotechnology and cancer research. But it was while developing rapid diagnostics for biological toxins at ASDx Biosystems, a company I started in 2012 in Boulder, Colorado, that I was asked by a headhunter if I'd be interested in designing, building and staffing a medical cannabis testing lab in Las Vegas, Nevada. Today, I am the Chief Science Officer at the lab I set up – Digipath Labs. Entering the cannabis industry, I immediately saw that there was a lot of false information out there, as well as a lack of transparency at the dispensary level. My motivation is to educate cannabis users, so they can make more informed choices about what they put into their bodies. Prior to the cannabis industry, my career has been long and extremely varied, spanning various disciplines and subjects, where I have always strived to bring an innovative perspective.

Some of the most common misinformation in the cannabis industry is related to strain naming. There's no agronomic convention used in naming cannabis strains, whereby you register your cultivar and establish verification criteria. The ambiguity allows for a lot of confusion – and even fraud. Thankfully, a number of influential groups are now calling for the industry to embrace standard naming conventions for cultivars.

"As cannabis enters the global marketplace and the path towards evidence-based medicine, standardized methods for cannabis testing are inevitable."

I wanted to see just how much diversity in cannabis strains there was, so we started doing large-scale data analytics based on the samples we are sent for testing. We are now working on "the terpene project", proposing a new classification scheme for cannabis based on terpene chemoprofiles. For one project, we looked at 2,200 individual flower samples, and across those samples there were 403 different strain names. By characterizing clusters based on their terpene chemoprofiles, we showed that there were really only three groups, and only 28 strains that could be called unique. Now, I'm working with scientists in other US states using the same in-depth data analysis.

As well as using terpenes to identify strains, I have a lot of interest in formulating terpenes, because I think they are a huge component in the "entourage effect." Also, there is more freedom to study the terpenes because they don't sit under that Schedule 1 stigma. As the cannabis industry matures and as we move towards evidence-based medicine, there is an absolute need for confidence and reproducibility in what people are growing.

I would like to streamline the required testing for cannabis – Nevada has the most rigorous testing requirements of any state, and there is some overlap in the tests we have to carry out. Cannabis is already a very challenging matrix to work with and the level of testing we do is very time-consuming and expensive. Certainly, no food product is tested to this extent in Nevada or elsewhere.

As cannabis enters the global marketplace and the path towards evidence-based medicine, standardized methods for cannabis testing are inevitable. Cannabis and cannabis-based products have to be reproducible, and I think we're going to move further away from the flower and more toward all-plant extracts. Cannabis presents many opportunities, so it's an exciting field to be in – if the federal regulations get relaxed, it's going to be amazing.

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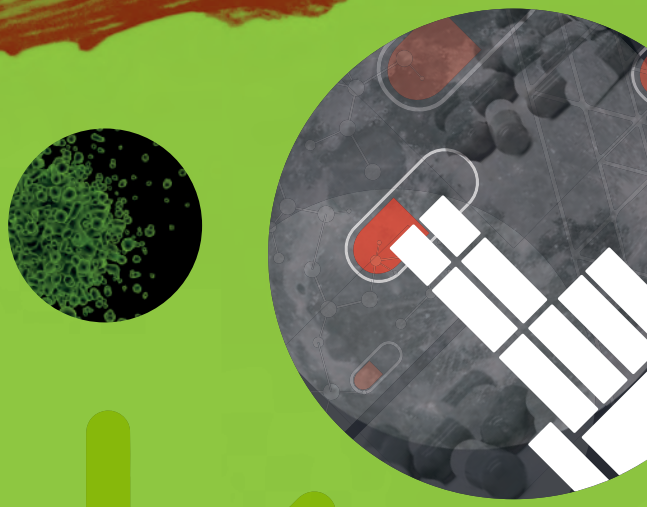

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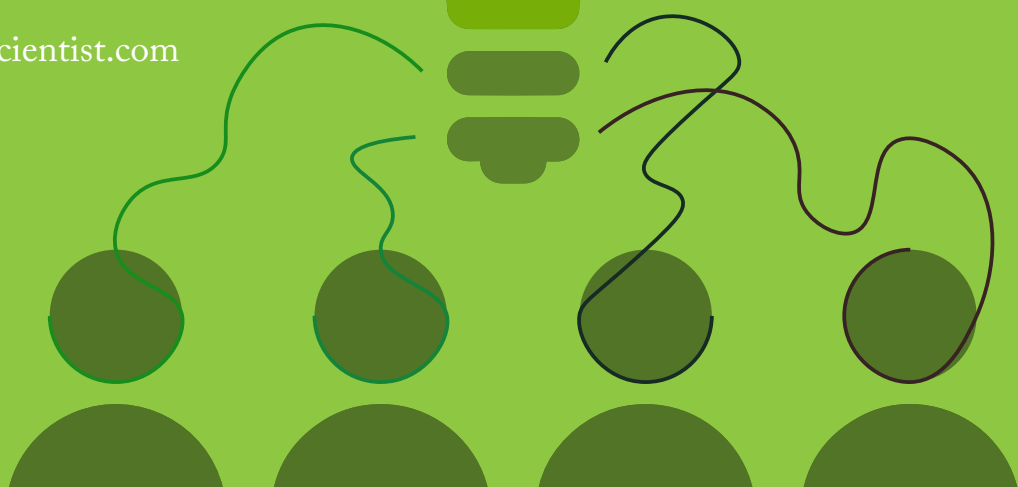

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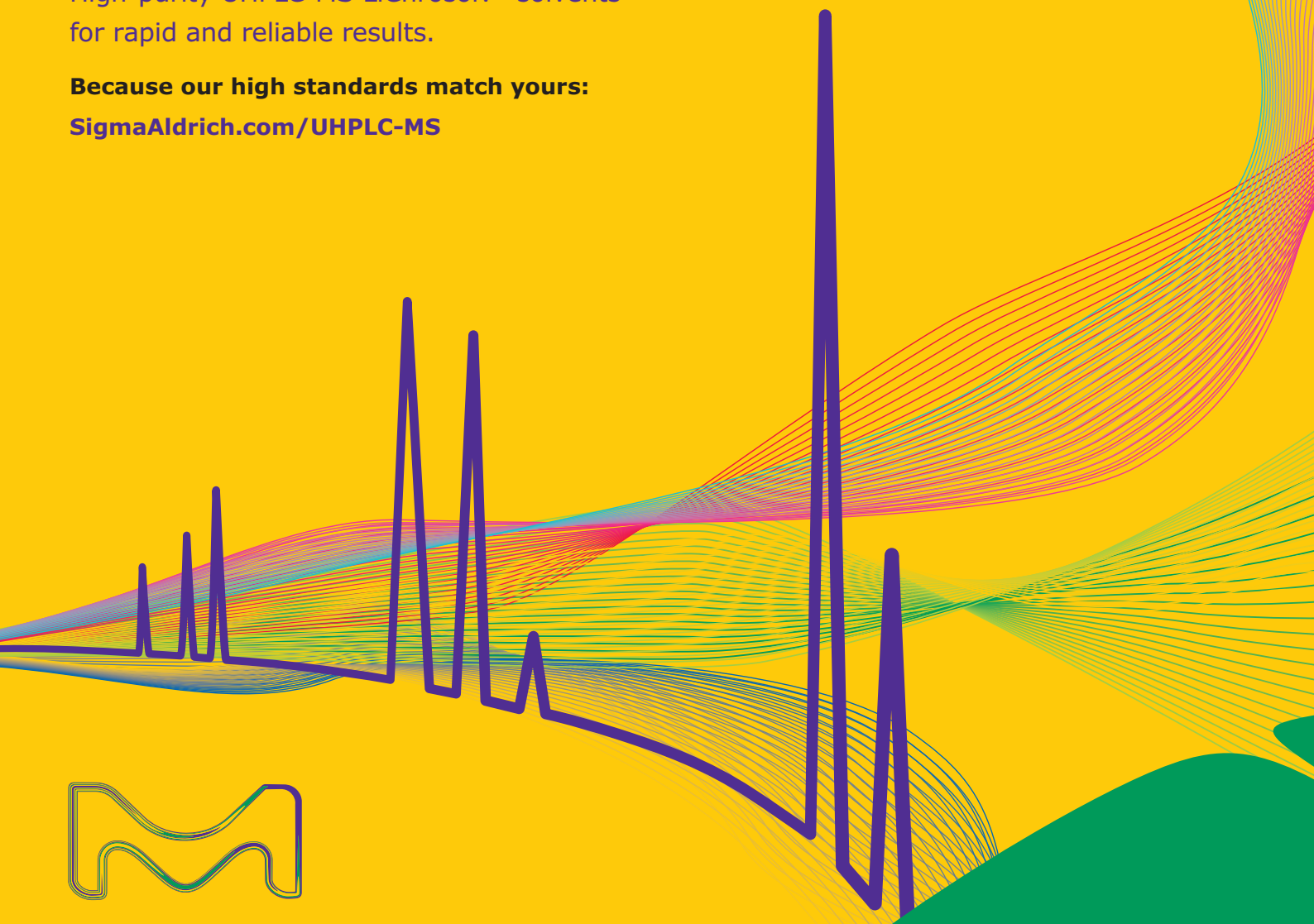

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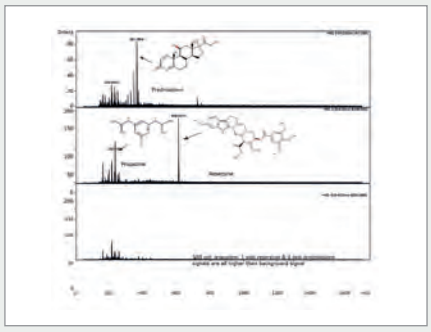
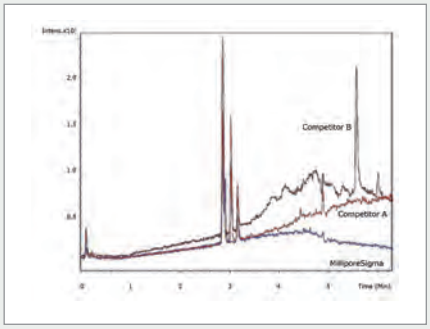


Test conditions: UHPLC-MS Gradient run with LiChrosolv® acetonitrile for UPLC- MS and LiChrosolv® water for UHPLC-MS from 2 percent acetonitrile to 98 percent acetonitrile. UHPLC column: Supelco Ascentis® Express C18, 2 µm, 2.1 x 30 mm. MS instrument type: Q-TOF. Detection: ESI (+).

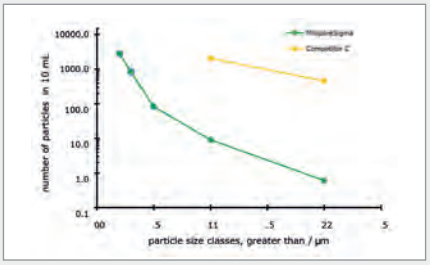


Test conditions: UHPLC-MS gradient run with LiChrosolv® methanol for UHPLC-MS and LiChrosolv® water for UHPLC-MS from 2 percent methanol to 98 percent methanol. UHPLC column: Supelco Ascentis Express C18, 2 µm, 2.1 x 30 mm. Detection: ToF-MS (ESI(+)).

Test 2

Comparison of LiChrosolv® methanol for UHPLC-MS (blue line) with two competitor UHPLC-MS products.

LiChrosolv® methanol for UHPLC-MS shows a flat baseline and by far the lowest impurity profile compared to the competition. Both competitors (high purity UHPLC-MS products A and B) show a baseline drift and significant impurity peaks.



Test conditions: The solvent samples were measured five times each by a single particle counting system (RION KS-40BF) based on light diffraction.

Test 3

Measurement of particle concentrations in LiChrosolv® acetonitrile for UHPLC-MS and a competitor UHPLC-MS grade solvent. The particle concentrations are divided into different particle size ranges. Competitor C displays significantly higher particle concentrations in all analyzed particle size ranges. Low particle concentrations prolong the lifetime of filters and mechanical parts in HPLC systems and reduce danger of column clogging. High particle concentrations might also result in detection of impurities.

RISE OF THE MACHINES

Found everywhere from smartphones to space missions, automation and artificial intelligence inspire awe and suspicion in equal measure. But what part could "smart" machinery play in the advancement of analytical science? We ask the experts whether intelligent instrumentation and automated workflows are a window of opportunity – or a threat to our future…

THE TIME IS NOW

Ready or not, automation is coming soon to analytical labs everywhere. Here's why we should rejoice in – rather than resist – the rise of the machines.

By Bob Boughtflower (Independent Consultant, UK) and Paul Hopkins(GlaxoSmithKline, Ware, UK).

In every industry, scientists typically follow set workflows to ensure that a product or formulation is prepared in a consistent manner. For analytical scientists, following methods and procedures provides confidence in the result that we generate. Depending on the sector, the application and the task, these workflows range from being somewhat intuitive to being completely locked down and controlled.

However, almost all of these workflows are subject to some degree of human interpretation or style that can introduce variations – either deliberately or unknowingly – into the output. Furthermore, external "noise" often has an influence and is, by definition, difficult to control. For example, a sonication step in a method may be subject to influences from many factors (water depth, water temperature, bath size, power, and so on).

In the analysis of valuable products such as pharmaceuticals, there is still a substantial element of manual processing involved, particularly in the preparation of samples. These processes are carried out within tight specifications or guidelines (according to where in the regulatory framework they belong), but variation may still be introduced. This could be due to differing interpretation of instructions, such as "stir until dissolved", or human error, such as recording a reading incorrectly. Any error that is introduced in the sample preparation will likely propagate and lead to variation or even failure of a process specification.

There is little doubt that most, if not all, of these "human" errors or variations could be eliminated by introducing automated processes to manage these tasks more reproducibly. Perhaps even more importantly, a machine can record and confirm the progress of the process to ensure compliance with the requirements or flag any issues before errors are propagated, providing assurance of data integrity. Of course, deliberate automation only becomes worthwhile when the process or product volume is high enough and well enough understood – or important enough to justify this level of investment.

As automation becomes more commonplace in the world around us, mankind continues to debate whether the increasing use of robots for some activities and jobs is a threat to society – perhaps our very future. Automating hazardous tasks is an easier sell, but using robots to improve reproducibility or eliminate non-value-adding, repetitive tasks is still met with suspicion from many – even in scientific circles.

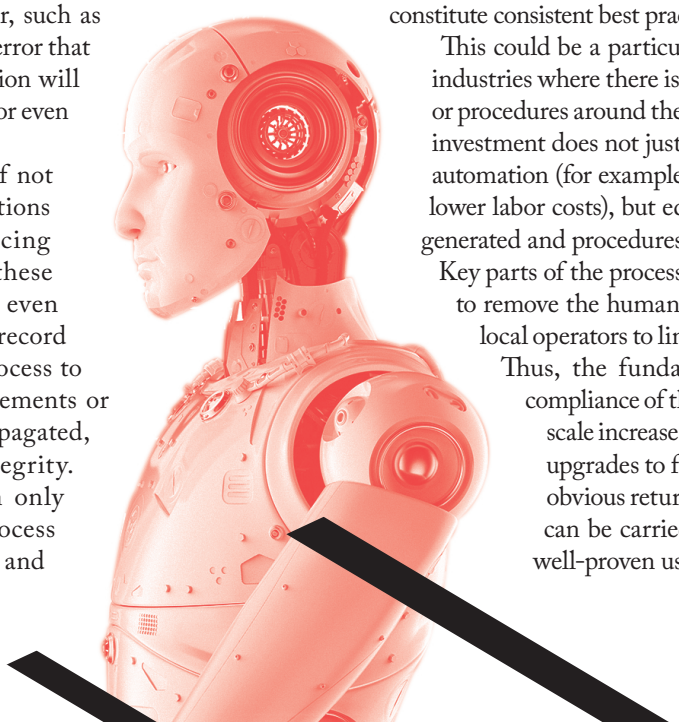
Opportunity knocks

So, should we embrace automation? For routine, set work flows, where there is a consistent throughput and a good return on investment, adoption of automation seems to be a sensible approach. The simplicity or complexity of automation should be dictated by need and indeed ambition – we do not advocate for automation for the sake of automation, but where it adds real value. However, this value may come in many guises and thus, we need to be more strategic in how we make this journey.

For example, even if many procedures never reach a scale sufficient to justify building a dedicated machine, introducing automation for key steps to ensure the most common errors are reduced or eliminated may still be valuable. If we're particularly clever, we can introduce these new technologies with one eye on the future automation of the entire process. This allows building sophisticated workflows from a series of core tasks such as weighing/ liquid dispensing/ dissolution and transfer to measurement devices. Where the need never arises or the volume levels are not met for full automation, the individual, optimized core task devices and procedures could still be used independently, and still constitute consistent best practice.

This could be a particularly beneficial strategy for industries where there is a need to transfer methods or procedures around the world, where the return on investment does not justify investment in full-scale automation (for example, due to low throughput or lower labor costs), but equivalent results need to be generated and procedures controlled.

Key parts of the process can be usefully automated to remove the human variation, while still using local operators to link these core tasks together. Thus, the fundamental performance and compliance of the process is maintained. As scale increases and productivity improves, upgrades to full automation have a more obvious return on investment (ROI) and can be carried out confidently based on well-proven use elsewhere.



"The very real benefits of automation will only become a commercial reality if enough buy in occurs from industry users and commercial vendors."

This approach also influences the design of automation, as it implies component, task-based modules that can be used on a stand-alone basis to perform a specific, otherwise error-prone task; or configured with other modules into systems to manage a more complex process. On the other hand, where it's unlikely that a task will ever be carried out in stand-alone mode, a more integrated (specifically configured) design can be considered as an expert system – but should still be capable of being linked to and transfer work to and from more sophisticated workflows. This also raises the stakes for commercial involvement to develop appropriate "off the shelf" automation that is interchangeable within a common platform.

All of these proposed approaches support identification and adoption of more standardized interfaces and formats for work preparation and execution; resulting in vendor agnostic instrumentation, seamlessly integrated and managed. Allotrope Foundation are pioneers of this journey (https://www.allotrope.org).

As technology continues to develop at a rapid pace, what were aspirational approaches are becoming a reality. Imagine a world where compliance is built in and machines become intelligent enough to handle errors and process data automatically. In fact, rather than aspirational, these become fundamental expectations of automation.

Automation could guarantee that a process was carried out correctly and progressed through every stage without incident, with all important parameters checked, monitored and recorded. Data integrity compliance comes as a standard feature. As the cost and complexity of monitoring and tracking existing processes in a fast-changing regulatory environment increases, the case for expert automation becomes ever stronger. Further developments in artificial intelligence and automated decision-making mean that automated release of products following testing is an achievable reality (see page 26 for more on artificial intelligence).

Time to answer?

The time is upon us where we can successfully automate many of the sophisticated processes that many still believe rely on the skill of highly trained human operatives and scientists to be successful. Well-designed automation will increase reliability, reproducibility, productivity, confidence and process compliance whilst decreasing error rates, time to decision, and – ultimately – cost.

We would like to see a more deliberate assessment of the potential for automating many tasks that make up key processes in the analytical science industry. In fact, workflows in many industries stand to benefit from this building block approach, which can be adopted as required as steps towards a long-term automation strategy – there are numerous examples of this happening in other industries, such as medicine and automotive manufacturing.

We need to think more strategically and innovatively about how we develop and ultimately deploy automation. Strategies need to be enabling – not just built around what is commercially available or accepting of an inefficient, non-robust "traditional" way of completing a task. Having a fit-for-purpose, scalable approach could remove some of the aforementioned barriers, and is likely to result in increased adoption.

All of the very real benefits of automation will only become a commercial reality if enough buy-in occurs from the user industry as well as the commercial vendors, who will need to work more collaboratively to develop co-operative automation (hardware and software). The science industry in general can be somewhat individualistic about the particular vendor or design of automation that is adopted, and there needs to be more collaboration around performance requirements and desirable standards. It is not entirely down to the big company end-users to each try and reach these goals – the companies that support them, such as CROs, are arguably the ones who have the biggest interest and throughput requirements in winning the contracts that make sense for the automation investment.

Change management also has a very important role to play here, and companies can afford to be much more transparent about which roles really require highly trained human staff, and what processes should intentionally be automated.

Transformative change is something that is often mooted. Maybe now is the time to deliberately replace out of date, inefficient, error prone processes with automated machines and systems able to industrialize laboratory and factory processes to a previously unimaginable level.

CONNECTED CHEMISTRY

How we're working towards the dream of a "smart" laboratory.

By Gurpur Rakesh D. Prabhu and Pawel L. Urban, Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan.

The technology geek at the heart of an analytical scientist is naturally driven to automate repetitive tasks performed in laboratories (1). As early as the 1950s, Leonard Skeggs introduced one of the first automated analytical techniques, continuous flow analysis (CFA) (2). In this technique, chemical reagents are introduced to the sample plugs, which move along a tube, separated with air bubbles. Chemical reactions occur as the train of plugs advances from the tubing inlet toward a detector. With this advance, variability of the analytical results became independent of sample processing by human analysts. Later, to address the limitations of CFA, flow-injection analysis was introduced (3), eventually leading to the development of microfluidic systems (lab-on-a-chip) (4).

Today, automated systems can perform high-throughput chemical analysis, as well as record and process experimental data more accurately and efficiently (5). With the introduction of artificial intelligence to chemistry research, analytical chemists can now obtain deeper insights from the colossal amounts of data recorded by the laboratory equipment (6). Algorithms assist in mass spectral interpretation and prediction, chromatographic peak picking, structural elucidation of molecules, reaction product prediction, experimental planning, and many more (7). Moreover, the open-source tools now available, such as microcontrollers, single-board computers, 3D-printing and software accessories provide great fodder to an analytical chemist who is hungry for innovation (1,8-10). Here at the Urban Laboratory at the National Tsing Hua

Platform	Advantages	Disadvantages
Continuous flow analysis	- analyses conducted in a series - simplicity - few mechanical elements	- technical issues with introducing/handling gas bubbles - certain sample treatment steps cannot be implemented
Flow injection analysis	- analyses conducted in a series - small volumes of solvents/reagents consumed - small volumes of chemical waste produced	- requirement to control dispersion of sample zones - sample/reagent mixing issues - certain sample treatment steps cannot be implemented
Microfluidic systems (lab-on-a-chip)/ micro total analysis systems (mTAS)	- portability - ultra-small volumes of solvents/reagents consumed - ultra-small volumes of chemical waste produced - multiple sample treatment stages	- appropriate expertise of operators required - microfabrication required - maintenance issues - dispersion/mixing issues - clogging microchannels
Microtiter plate-compatible systems	- analyses conducted in parallel - compatible with many standard assay procedures - compatible with many conventional detection systems	- large volumes of consumable materials required (plates) - large volumes of solvents/reagents required - large volume of solid and liquid waste produced - limited flexibility
Centrifugal analyzers	- analyses conducted in parallel - reduced handling of liquids	- bulky - only for specific assays (limited flexibility) - require the use of designated cartridges with solid and liquid materials - large volume of solid and liquid waste produced
Cartridge analyzers	- all-in-one solution for a few standard assays - portability - simplicity of use	- only for specific assays (limited flexibility) - require the use of designated cartridges with solid and liquid materials - large volume of solid and liquid waste produced
Autosamplers/robotic systems with restricted movement	- relatively robust - easy to program	- mechanical complexity - limited number of analytical procedures that can be executed - bulky - involve multiple mechanical operations
Multi-axis robots	- high degree of freedom - high flexibility	- mechanical complexity - high cost - safety concerns - complex maintenance - large size - involve multiple mechanical operations
Total laboratory automation	- high throughput - flexibility for a large number of programmed procedures - reduction of personnel costs	- high mechanical complexity - high cost - complex maintenance - very large size - involve multiple mechanical operations

Table 1. Advantages and disadvantages of some of the main automation approaches. Adapted with permission from (1).

"With the introduction of artificial intelligence to chemistry research, analytical chemists can now obtain deeper insights from the colossal amounts of data recorded by laboratory equipment."

University, Taiwan, we make use of these inexpensive open-source tools to automate our analytical procedures.

For example, in one study, continuous and automated dilution of complex samples was achieved using an Arduino-based control unit by plug-volume modulation, a technique involving continuous introduction of short plugs of a sample, separated with short plugs of a solvent (11). Automated one-shot multipoint calibration of analytical detectors is possible with this simple setup. Another project led to the development of a dual robotic arm "production line" for sample processing and introduction to a mass spectrometer (12). The robotic arms allow the analysis of multiple samples without human interaction. Moreover, a system built around a miniature single- board computer was used to determine optimum sample flow rates for mass spectrometric analysis (13). The system – referred to as a sample flow rate scanner – can also be used to determine the detector sensitivity regime. Recently, an automated sampling system was developed to enable mass spectrometric analysis of volatile organic compounds emitted by live biological organisms (everything from microorganisms to mushrooms) (14). The sampling system can help scientists draw insights from the dynamics of volatile compounds released by biological organisms – for example, in response to external stimuli.

The analytical chemist's dream of a "smart laboratory" is not far from reality. The "Internet of Chemical Things" (15) can now connect, interact and exchange data among various laboratory equipment – keeping analytical chemists, chemical manufacturers and technology developers interconnected.

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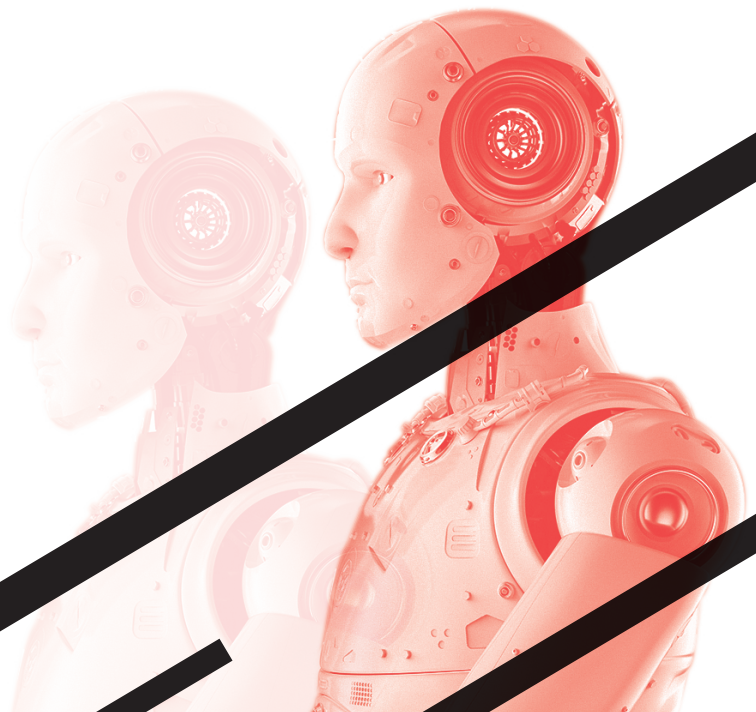


DO ANDROIDS DREAM OF ANALYTICAL CHEMISTRY?

We ask Peter Harrington, Director of the Center for Intelligent Chemical Instrumentation at Ohio University, whether artificial intelligence can help solve the field's big data challenges.

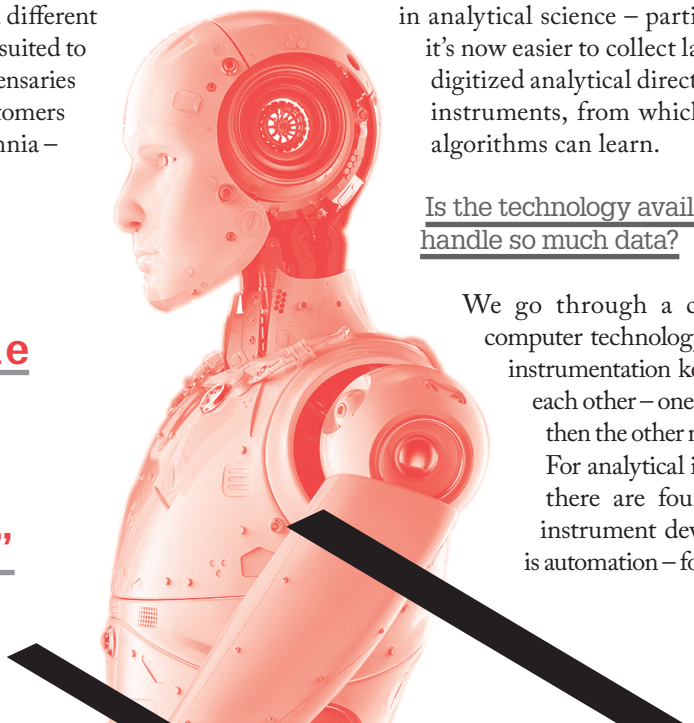
What is the current focus of your work?

We are working on spectroscopy and mass spectrometry analyses of botanical medicines, specifically cannabis. Our goal is to take the complete spectrum of a complex mixture, and relate that fingerprint to the chemical and pharmacological properties of the sample – known as chemotyping. To develop these complex algorithms, we use machine learning (also known as artificial intelligence).

One of the biggest challenges with natural medicine is biological variability. Everything from the growing conditions to the harvest date of the plant to how the plant material has been processed can affect the chemical composition; even samples from the same plant may have different properties. By chemotyping a sample, we get a snapshot of the overall chemical composition, which we can tie (directly or indirectly) to the properties that we're interested in, such as physiological effects in the body. The alternative approach is to try to identify every compound (or the active compounds) in the mixture and quantify them, but that's very time consuming and costly.

Longer term, we would like to see cannabis companies using the chemotypic data we're generating on different strains to help direct people to the product best suited to them. At the moment, the staff in cannabis dispensaries make personal recommendations to their customers – this strain for migraines; that strain for insomnia – but there's no scientific basis; it's all anecdotal. We'd like to get the science back into that decision-making process. Keeping a history of each consumer's experiences and correlating it with the chemical compositions of their chosen products would allow us to direct them to the products that they personally might find most effective. This kind of profiling might be applicable in the wider pharmaceutical industry too, where there's still often a "one-size-fits-all" approach to usage and prescription. As people's lives become increasingly digitized, we can use predictive algorithms to look for correlations between specific drugs and their effects on consumers.

"As cooks know all too well, the success of a meal often depends on the quality of the ingredients – and machine learning depends on the quality of the data."



How are you using AI?

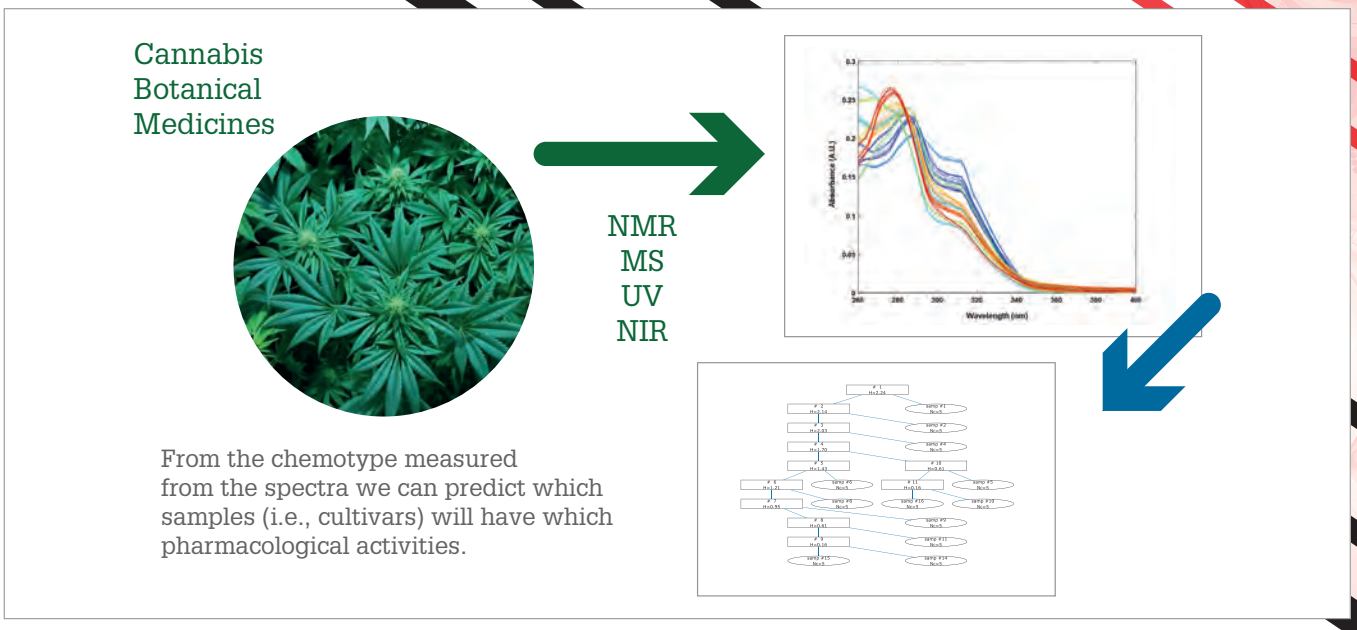
Simple artificial neural networks have been used since the earliest computers were developed in the 1940s. Later, multiple layers of neural networks were combined to boost learning power – known as "deep learning". We can see deep learning in action every day in applications like Apple's Siri or Facebook's face recognition feature.

Once the layers of neural networks get very deep, it becomes very hard to determine how the decisions are being made – in our case, to determine the relationship between the chemical signal and the property that we want to measure. To correlate peaks with properties, we need to be able to peel back the layers. I developed an Enhanced Zippy Restricted Boltzmann Machine (EZRBM), a modification to the RBM, developed by machine learning pioneer Geoffrey Hinton so that we can reveal the input variables (e.g., spectral or chromatographic peaks) that correlate with the network outputs (e.g., flavor, effect, age, provenance, etc.).

AI has a number of applications to problems in analytical science – particularly because it's now easier to collect large amounts of digitized analytical directly from modern instruments, from which the computer algorithms can learn.

Is the technology available to handle so much data?

We go through a cycle in which computer technology and analytical instrumentation keep leapfrogging each other – one pulls ahead, and then the other needs to catch up. For analytical instrumentation, there are four goals driving instrument development. One is automation – for instance, using



Our approach to identifying the chemistry behind specific pharmacological traits in cannabis.

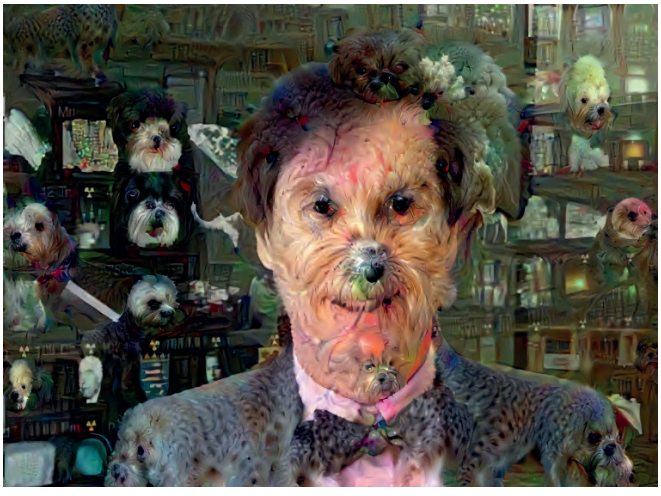
robotics or microfluidics for sample preparation and autosamplers that can allow instruments to generate data continuously for 24 hours a day, seven days a week. The second is resolution (more data per measurement), the third is speed (faster measurements), and the fourth is lower detection limits, which increases the complexity of the data because features from components that were previously below the detection limit will become detectable. All four lead to burgeoning streams of data that are amenable to machine learning.

Then we have advances in computer technology. At the moment, these center on increasing our storage capacity and computational efficiency. Twenty years ago, I gave lectures in which I tried to explain the number of connections in the human brain – about one terabyte – by saying that you would have to connect 1,000 one-gigabyte hard drives to obtain the technological equivalent. Nowadays, we can buy 10 TB hard drives for a few hundred dollars! In addition, clusters and chips are available with many individual processing units that can all operate in concert.

The next issue to tackle is locating data on a drive that size. That's also improving; our scan rates are steadily increasing. So, whenever a scientist tells me that their computer is not large enough or fast enough to handle these large volumes of data, I say, "That may be true now, but in two or three years, it is likely to be another story."

What are the limitations of AI?

I find the rapid development of machine learning exciting – but people don't always agree. They ask me, "Should we be afraid of advances in AI?" I don't think so, although it's not unreasonable to consider the disadvantages. Because these systems are so complicated, when things go wrong, they tend to go terribly wrong. And once they've gone wrong, they can be hard to fix, because many networks are inscrutable. And there are other limitations; machine learning is just an algorithm that follows a procedure – it is not "intelligence," just as a recipe is not a cook. A human being must still write that algorithm and connect it to the data; we have yet to reach the point where computers can program themselves. As cooks know all too well, the success of a meal often depends on the quality of the ingredients – and machine learning depends on the quality of the data. They also can't deal well with extrapolation or novel data. You may have seen "computer dreaming," which is a form of digital art in which you train a system using a series of images (for example, of dogs) and then provide them with new input (such as a picture of a human). The algorithm attempts to build a picture of a human using what it has learned about dogs, which, as you can imagine, is quite surreal. So the technology is not without its problems, but I don't think we have anything to fear at least at the moment!



Google's Deep Dream Generator (https://deepdreamgenerator.com) – trained on images of the author's dog – attempts to render his picture, with surreal results!

GURUS OF

Three connoisseurs of capillary electrophoresis – Hermann Wätzig, Cari Sänger-van de Griend and Michael Breadmore – discuss the current state of the art, and what's coming down the tube.

What are the benefits of CE?

Hermann Wätzig: It is quite simply the best separation technique for biomolecules. It is precise yet straightforward, provides excellent separation efficiency and allows many options to tune selectivity. Gel electrophoresis (GE) is far more difficult to use for quantitative analyses, and chromatography will always remain something of a black box due to the huge number of intended and unintended interactions possible (silanols, size-exclusion effects, changes in selectivity from adsorbed matrices, to name just a few). There are many interactions on capillary walls too, but these fused silica surfaces are much simpler than the spherical particles of differing size and surface properties involved in chromatography. Every major bioanalytical lab needs CE at least as a reference technique. Bioanalyses are always fairly complicated, and it's easy to misinterpret the resulting data; therefore, it is essential to not only obtain a good hypothesis using one approach, but also confirm it with a second technique with different properties.

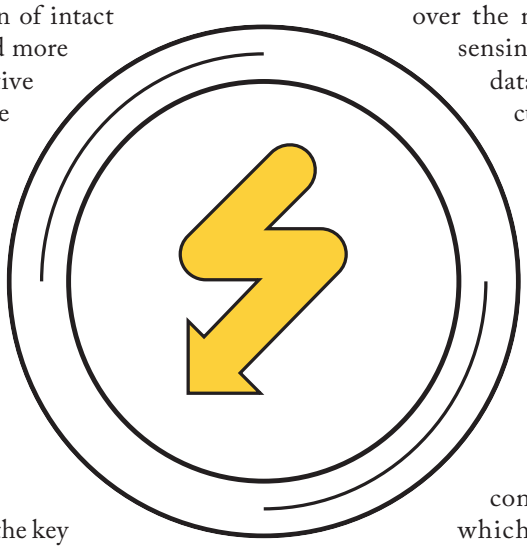
Cari Sänger-van de Griend: Electrophoresis is more than 200 years old, and from its inception has demonstrated tremendous separation capability. CE combines speed, quantitation, reproducibility, robustness and automation with the fundamental high-resolving powers of electrophoresis. In pharmaceutical analysis this means an additional technique that is fundamentally different from – and therefore complementary to – chromatography.

Michael Breadmore: Electrophoresis is wonderful for speed and simplicity. The speed is unmatched by chromatography so CE is a great option when time is important (such as for high-throughput or near-real-time applications) or when you can't afford the time to send a sample back to the lab. Our work on airport screening for inorganics (and the commercial product, GreyScan) is a perfect example of where you can do with electrophoresis what you cannot do with chromatography or mass spectrometry (MS). Electrophoresis has always been able to separate macromolecules and particles in a way that LC cannot, and as we grow increasingly concerned about the potential health and environmental impacts of nanoparticles, the value of electrophoresis to separate and analyse these will become more evident. In the biological realm, electrophoresis may offer some quite unique capabilities for cells, exosomes, organelles, and perhaps even organisms.

What are the key applications?

CS: The impact of CE is often underestimated – the Human Genome Project could never have finished so early without the CE at the core of every modern DNA analyzer, and capillary gel electrophoresis (CGE) and capillary isoelectric focusing (cIEF) are part of almost every biologic license application (BLA) submitted today. In small-molecule pharma, several companies have used or still use chiral CE as a technique of choice.

Recently, we developed a capillary zone electrophoresis (CZE) method for the determination of intact virus particles (1), which is faster and more precise than the existing quantitative PCR methods. There are many more examples like this from industry, but usually they remain unpublished.



MB: Pharma and clinical diagnostics are the key commercial strongholds at the moment. It also presents some important capabilities in forensics, and I have hopes it will penetrate into airport screening, but it's still early days for this application.

HW: I agree that pharmaceuticals are the key application. The major field of application within the pharma industry is monoclonal antibodies, including biosimilars and antibody–drug conjugates. However, CE is making inroads in areas such as vaccines. These can be very complex mixtures of biomolecules and require the best separation efficiency you can get.

Where might electrophoresis be applied in future?

CS: The strengths of electrophoresis techniques can be further exploited in areas such as palm-top/handheld instruments. These would bring the lab to the sample, and could be used in home diagnostics, counterfeit detection, explosive detection, quality control in low-resource countries, and more. I would say "the sky is the limit" but I read a paper recently about CE being sent into space!

MB: I believe that electrophoresis will have real impact over the next few decades in environmental sensing – providing spatial and temporal data about our environment that we can currently only dream of. This rich seam of data could inform climate models, increase agricultural productivity while minimizing environmental damage, and provide new insights into the environmental significance of nanoparticles. I also believe that electrophoresis will form the basis of a generation of point-of-care sensors for urgent health applications.

But even this is thinking quite conservatively. One of the areas in which electrokinetics has traditionally been overlooked is sample preparation. Chromatography (liquid–liquid extraction and solid-phase extraction) is the current mainstay, but recent work has shown the potential to purify molecules from complex samples with electric fields – there are even reports of soil remediation with electric fields.

“I believe that electrophoresis will have real impact over the next few decades in environmental sensing – providing spatial and temporal data about our environment that we can currently only dream of.”

HW: As well as the new avenues that Cari and Michael describe, I see many further possibilities in areas where classical electrophoresis has been and still is used. The many possibilities for tuning selectivity in CE have not been fully employed for protein analysis – for example, using cocktails of reagents to separate physicochemically similar species, and engaging affinity CE.

What recent developments could lead to wider use of CE?

MB: Improvements in electronics and computational technology will continue to have a considerable impact. High-voltage power supplies as big as your thumb but costing less than US$100 are now commercially available, and open-source controllers (such as Arduino boards) and small processors (such as Raspberry Pi) will enable instrumentation that is dramatically smaller and cheaper. The small CE developed by Mehdi Moini at George Washington University, which clips on the front of a mass spectrometer, is just one example of what might

GURUS

Cari Sänger van de Griend, Kantisto

My focus is on the analysis and characterization of drug substances, products and excipients for small molecules and biopharmaceuticals. Having worked for many years in the pharmaceutical industry I launched my own consultancy (Kantisto) in 2011, which gives me the opportunity to focus the topics I find most interesting – including capillary CE! My work now consists partly of consultancy, training and collaborations in pharmaceutical analysis and regulatory science, and partly CE-specific troubleshooting, R&D and training. Recently, Kantisto joined an exciting Horizon 2020 Innovative Medicines Initiative project, iConsensus. Our task is to develop CE methods for real-time detection of reagents and quality attributes in a bioreactor.

GURUS

Michael Breadmore, University of Tasmania

I completed my PhD in electrophoresis in 2001 and have spent the last 15 years working on electrokinetic systems as a research fellow funded by the Australian Research Council. Electrophoresis (as a subset of electrokinetics) has always been seen as a replacement to liquid chromatography (LC), but in fact it's very different. My research is focused on trying to exploit those differences to maximum benefit – not to replace LC, but to do what LC cannot. The GreyScan technology developed by my group leverages the speed and accuracy of CE for airport explosives detection. Our ultimate goal is to develop a μTAS – a holy grail portable sample-in/answer-out instrument – for a range of clinical, environmental, security and industrial applications.

be still to come. This also suggests that CE may become more of an "add-on" to other analytical techniques such as MS, or indeed LC.

HW: Reliable instrumentation will bring about a much broader acceptance. Since the rise of modern CE in the late 20th century, the separation power of CE has been excellent, but high failure rates continued to blight the field. It is only in the last few years that reliable instrumentation has become available, with failure rates as low as that of HPLC.

Miniaturization is another development that has brought great advantages – increasing speed by at least one order of magnitude. Typical CE analysis times are in the range of a few minutes, but doing CE on microchips brings the time down to less than a minute – and often just a few seconds. This is particularly attractive if you have a very high number of samples or if you need to survey a process with high temporal resolution.

CS: Ongoing research in the fields of in-capillary concentration techniques and coupling of MS detection to the CE are leading to some real breakthroughs. Miniaturization creates opportunities for more automated sample prep, but could also be an opportunity to overcome some of the fundamental flaws in current instrumentation. For example, electrophoresis is a bi-directional separation technique, but all current instrumentation is uni-directional. So potential is wasted from the start.

Affinity CE has shown great promise form the early days of the field. Slowly, the biotechnology industry is catching on, but needs support in translating the concepts to methods. Personally, I would be thrilled to investigate

"Since the rise of modern CE in the late 20th century, the separation power of CE has been excellent, but high failure rates continued to blight the field."

the potential of CE for potency assays as I believe this would greatly enhance precision and speed up methods.

One of the most important developments in the implementation of CE is the identification and understanding of Good Working Practices for CE. This is a community effort and the transfer of this knowledge to all users is of major importance for the implementation and future of the technique.

HW: Recently, I have seen less competition between CE and LC; the real competitor is GE. However, with its superior quantitative abilities, CE can compete in many fields; for example, I have seen a lot of protein separations by size shift from SDS-PAGE to CE-SDS. In general, many labs have switched from GE to CE for antibodies and I expect to see the same trend for other proteins.

How does CE-MS compare with LC-MS?

MB: From what I have seen, CE-MS has the potential to be much more sensitive than LC-MS. The work of Norman Dovichi in the USA is leading the charge on proteomics and showing some exceptional coverage, while Tomoyoshi Soga in Japan and Govert Somsen in the Netherlands are doing the same for metabolomics.

CS: CE-MS and LC-MS are complementary – in pharmaceutical analysis in general and biopharma in particular, we need all the tools we can get. After all, protein characterization is a tough job! All the molecules are unique, so for different molecules (even ones as similar as mAbs), different techniques may be optimal. For example, when characterizing protein charge heterogeneity, the best technique could be ion-exchange chromatography, cIEF or CZE. The is no such thing as one-size-fits-all, although managers and kit-vendors like to believe differently.

HW: Certain separations are simply harder to achieve by LC, such as charge variants of proteins. New interfacing approaches allowing higher sensitivity in the presence of ampholytes in the separation buffer (Christian Neusüß´s group are doing excellent work here) should extend these advantages in future.

Where is the field heading?

CS: The future growth of CE is obvious in some areas and hopeful in others. In the biopharma industry, there is an increasing wish to complement the traditional CE-SDS and cIEF with the additional separation power and speed of CZE. However, the current CE-MS methodologies require MS-friendly CE buffers, and all too often MS-friendly means CE-unfriendly, with electrolyte ions such as ammonium leading to severe electromigration dispersion. I realize it is a big ask, but I challenge those working on the hyphenation of CE with MS to find solutions to make truly CE-friendly electrolyte solutions possible.

In the longer term, I hope that projects like iConsensus result in analytical platforms where CE is embedded in process analytical technology modules. Plus, there is an urgent need for advanced methodologies that are cheap, reliable and transportable for the regulatory and quality control of drugs in underdeveloped and developing countries, and CE is very well suited for the task.

GURUS

Hermann Wätzig, Technische Universität Braunschweig

I'm motivated by my curiosity – and the desire to pass this spirit of curiosity to others. CE has been one of my key areas of focus for many years, along with analytical quality in pharma. In particular, my group concentrates on improving CE for protein assays and purity control; a lot has been achieved in this area but there are still many improvements to be made. More recently, my group developed techniques for protein QC and ligand binding assays – the latter motivated by the success (and fun!) we have had using affinity CE.

"Scientifically speaking, we have resolved many good working practice issues, but many of the less 'sexy' areas need more research."

HW: I would not be surprised to see more affinity CE, an area that has been steadily growing in the last years. Affinity CE is an excellent reference technique for other ligand-binding assays, allows affinity estimation for weakly binding systems, and does not require pure substances so it is particularly well suited to early drug optimization.

MB: Perhaps I am too pessimistic, but I think the "golden days" of electrophoresis are gone, though I see it continuing to be the method of choice for very specific applications. That said, "separation sensors" is a space where I think electrophoresis will outcompete chromatography, and if systems fulfil their promise to provide near real-time information about the individual, the environment and the connection between the two, the demand for electrophoresis may end up eclipsing anything seen to date.

What are the greatest challenges for research in the field?

HW: The challenge is to boldly continue what has been started in the last few years: improvement in instrumentation and support. It would be great to see more versatile instruments maintaining the same reliability.

CS: One challenge lies within the pharma industry. Managers are always looking to reduce method development times and embrace one-size-fits-all platform solutions. The problem is that the unique physical–chemical and clinical properties that make an effective drug mean it's unlikely to show uniform properties in our analytical techniques, so we are constantly challenged to develop

new methods. That task is made easier for LC by the stream of emails I receive from vendors about LC applications and new columns, but useful application notes for CE are rare. In LC, competition has resulted in more robust equipment and better transferability between different types of LCs, as well as compatibility of different brands of columns with the majority of instruments. We still need to go through this process with CE equipment.

Scientifically speaking, we have resolved many good working practice issues, but many of the less "sexy" areas need more research. For example, there are several publications on the reduction of adsorption of proteins to the capillary wall, but there is still no consensus.

For the regulatory acceptance of any technique, multi-site studies are important, so we need strong collaboration between companies, academia and vendors, to identify both strengths and weaknesses of a method.

And last but not least, we should not underestimate the need for training. Currently, students learn surprisingly little about CE and its use in industry. Good training at universities and in industry is paramount, and needs to cover fundamentals, method development and good working practices.

MB: A challenge for all researchers, not just in this field, is the change in how research funding is allocated. Many academics want to work on "blue skies" research that may take decades to demonstrate value. But funding agencies around the world are increasingly focused on solving specific problems, with little money available for curiosity-driven research. Indeed, researchers are being driven to solve not the most important problems, but those that are financially viable. I believe we need to fund more basic research, and invest more to support translation outside of academia.

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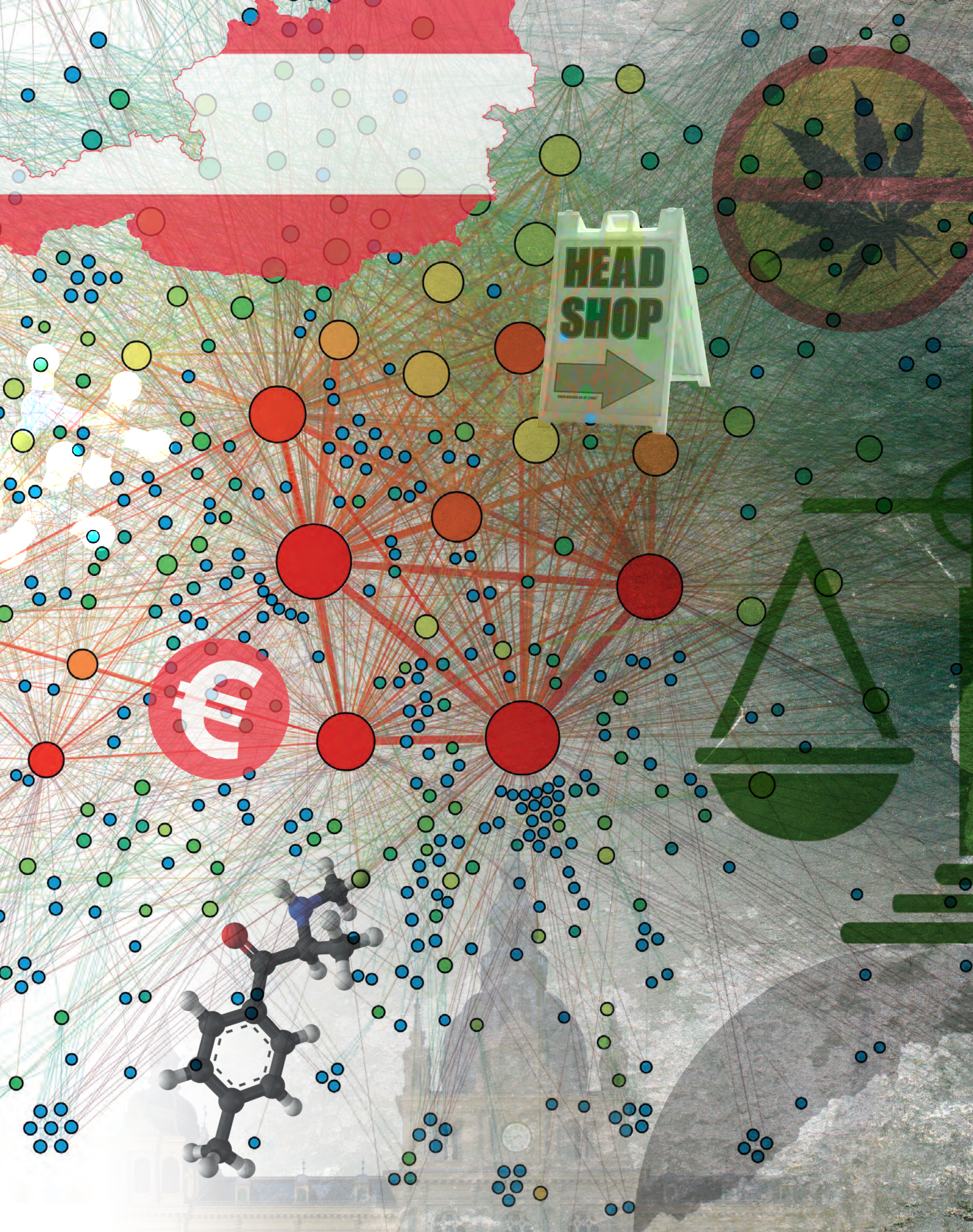
Analyzing the Underworld: *a Legal High*

How a respectable chiral separation scientist got sucked into the weird, wonderful, and unpredictable field of novel psychoactive substances.

By Martin G Schmid

Almost a decade ago, I'd never really given a second thought to how the scope of my analytical research might change in the future. But change it did!

Back in 2010, I was a senior scientist at Graz University. I developed chiral separation methods for active pharmaceutical ingredients, using various high-performance separation techniques, including HPLC, GC, capillary electrophoresis, and capillary electrochromatography – all highly respectable. My former supervisor was retiring, and I was charged with taking over his lessons. I felt that one of those lessons – the analysis of illicit drugs – could do with a little more "spice." But how?


HEAD
SHOP

Hello, officer!

I visited the main police station in Graz and asked if there was any interesting news about abuse of drugs in public parks that I could share with the students; I wanted to ground the lesson in reality. I received a warm welcome – and several interesting stories about dealers – from one police officer. But if it were not for the presence of a second police officer on that day in early March 2010, my scientific field of interest would not have changed so dramatically. This enthusiastic character was excited to ask me if I had heard about "novel psychoactive substances" (NPS) – also known as Legal Highs, Research Compounds, Leisure Drugs, Plant Food, Bird's Cage Cleaner or Room Odorizers…

The compounds were traded via the Internet or in so-called headshops, he told me, but they were not scheduled by Austrian law, making them a special challenge for the authorities. Because national labs were overloaded, analyses of these tricky samples were often considerably delayed – and dealers were being wrongly imprisoned, given that the compounds could actually be traded legally. The police officer also tipped me off on the name of a compound that came up again and again over the following years: mephedrone (4-methylmethcathinone). After assuring him that I would look into this challenging but exciting situation, I was faced with my first problem. How does one go about procuring even one gram of such compounds? When faced with an unanswerable question, I did what everybody does: I turned to the Internet. A quick search pointed me to a source of mephedrone in the UK.

And so I found myself entering into a whole new world with my first purchase of a psychoactive substance. Three days later, my order landed in my mailbox (at home!). I was eager to perform a GC-MS analysis and – somewhat unexpectedly – I discovered that my first sample was of the same purity as denoted on the label. Next, I elucidated the mass spectrum of the compound and, suddenly, I was in a position to help identify mephedrone in samples seized by police.

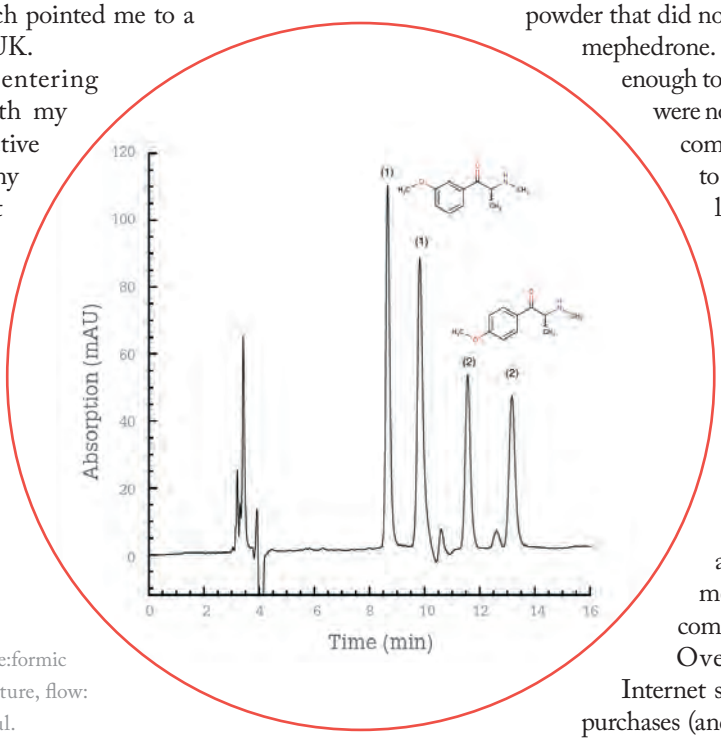


Figure 1. Simultaneous enantioseparation of (1) 3-MeOMC and (2) 4-MeOMC. Column: Lux Cellulose-4, 250x4.6 mm, 5 µm; mobile phase: Acetonitrile:isopropanol:diethylamine:formic acid (95:5:0.1:0.1); ambient temperature, flow: 1ml/min, UV: 254 nm, injection: 5 µl.

But I was still intrigued – there was more to learn; mephedrone possesses a chiral center, which encouraged me to develop a chiral separation method to prove that it was being sold as a racemic mixture. I was not surprised when I saw two peaks in my chromatogram (see Figure 1) – after all, why should a novel psychoactive substance be traded as a pure enantiomer, when achieving such purity would be a cost-intensive process? That said, at that time, nobody knew which of the two was the eutomer (the enantiomer with the desired pharmacological activity).

As an aside, I was actually very lucky with my first drug purchase; some days later, the law was changed in the UK and mephedrone was banned overnight. As a consequence, many Internet shops were no longer allowed to trade the compound. Meanwhile, a headshop in Graz continued trading mephedrone, apparently serving a long line of customers every weekend. Towards the end of 2010, the first flood of NPS hit the whole of mid-Europe.

A growing collection

I continued to perform analysis for the police, distinguishing between established illicit drugs and NPS that were still legal at the time. At some point, the police showed up with a new powder that did not match the mass spectrum of mephedrone. One of my colleagues was kind enough to record an NMR spectrum, we were now in possession of a brand-new compound that was closely related to mephedrone. The name of this latest designer analogue was 3,4-dimethylmethcathinone (3,4 DMMC) and its very existence hardened my resolve: I would collect as many NPS samples as possible! Specifically, I wanted to know if all such compounds were traded as racemic mixtures and, at the same time, gain knowledge and develop chiral separation methods for these little-known compounds.

Over time – and with many Internet searches and more than a few purchases (and failed purchases) – I became

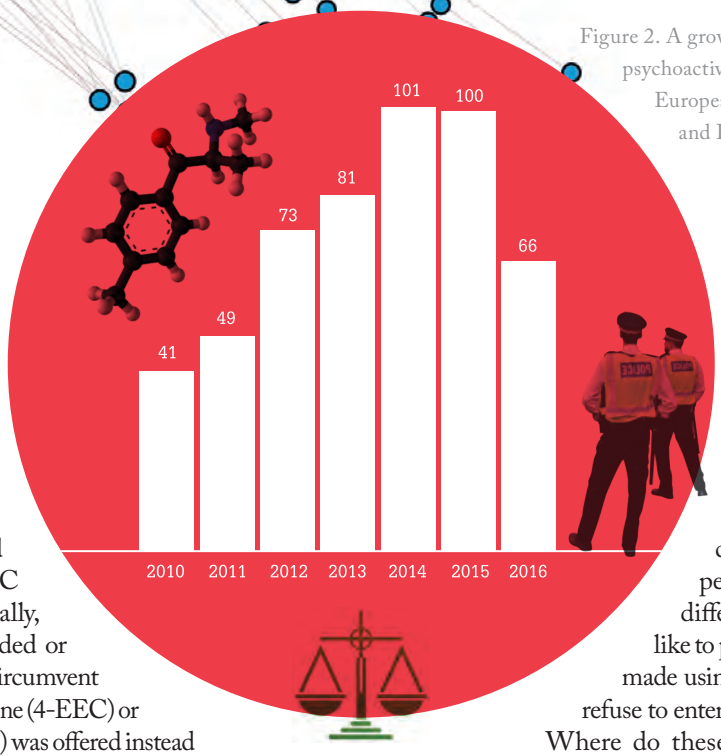


Figure 2. A growing problem: number of novel psychoactive compounds reported to the European Monitoring Centre for Drugs and Drug Addiction each year.

increasingly familiar with the world of NPS and its (short) history. Essentially, I could see rapidly shifting trends as they happened. After the ban of mephedrone, it was immediately replaced by some closely related compounds, the most prominent of which were the aforementioned 3,4 DMMC and 4-MEC (4-methylethcathinone). Typically, a methyl group was either added or replaced by an ethyl group to circumvent the law. Later, 4-ethylethcathinone (4-EEC) or 4-ethylmethcathinone (4-EMC) was offered instead of 4-methylethcathinone. In parallel, halogens were introduced into the phenyl ring of mephedrone yielding 4-fluoromethcathinone (flephedrone, 4-FMC), and 4-bromomethcathinone (brephedrone, 4-BMC) and 4-chloromethcathinone (clephedrone, 4-CMC). Interestingly, all these compounds were first available in their para-form, followed by meta- and ortho isomers. Methylmethcathinone turned out to exhibit six possible forms: 4-MMC, 3-MMC, and 2-MMC each with two enantiomers.

Eight years later, my NPS collection contained 75 of the approximately 120 known cathinones. All of them were designed to circumvent the law and still little is known about their main effects, side effects and long-term effects. And that's probably why, seeking some clue as to what to expect, NPS consumers read about the experiences of arguably less-than-trustworthy users on Internet forums…

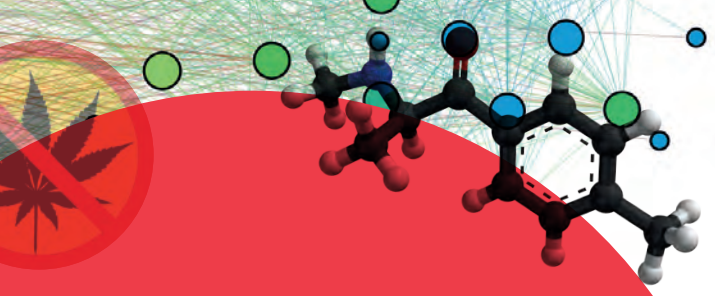
As another aside, I'll note that simply growing my collection of hundreds of samples was an interesting endeavor. Notably, prices vary wildly; on Internet platforms 1 g of a compound costs about 20 euros, but through traditional vendors (for example, LGC Pharma) 10 mg costs over 125 euros! In other words, even if I encounter many Internet "scammers" who take my money, but fail to provide the right (or any) substance, overall it's still cheaper to buy online – assuming you have the analytical capability to check you've got the right compound. To that end, after a successful purchase, my samples were identified by GC-MS and, if necessary, by NMR. Interestingly, about 70 percent of my purchases resulted in the correct compound – the other 30 percent? I either got something different or nothing at all. I'd also like to point out that all purchases were made using the "Clearnet" – I absolutely refuse to enter the Darknet!

Where do these huge volumes of so-called "Research chemicals" come from? Well, they can be ordered at the kilogram scale – and they are often manufactured in China. There, hundreds of brilliant chemists wait for orders to synthesize compounds according to a given chemical formula. As far as I know, these people are not always aware of how the compounds will be used down the line.

Novel compounds, novel methods

So, what have I been doing with my collection? I suspect something quite different to most regular buyers! At first, my goal was to build up a GC-MS database of novel psychoactive substance. Next, all chiral compounds were subjected to enantioseparation using various high-performance techniques. Over the years, I've tested many different chiral HPLC columns, partially in normal-phase but also in reversed-phase or polar-organic mode. Here, cellulose or amylose-based chiral stationary phases turned out to be a particularly good choice. We were not quite so successful with cyclodextrin-bonded chiral phases; however, the use of ß-cyclodextrin derivatives as

Figure 3. Ingenious and clandestine packaging used to deliver NPS.



Scheduled drugs	NPS replacements
Cannabis	Broad spectrum of synthetic cannabinomimetics
Amphetamines and mephedrone	New bath salts
Cocaine	4'fluorococaine
Ecstasy	Methylone
LSD	Legal tryptamines, highly effective new phenethylamines, and other LSD-analogues
Methylphenidate (Ritalin)	Ethylphenidate

Table 1. Any classical scheduled drug can be replaced by NPS.

mobile phase additives showed positive results both for RP-8 and RP-18 phases (1).

With gas chromatography, the main emphasis was on indirect chiral separation. After sample pretreatment with different chiral derivatization agents, several racemates were resolved as their diastereomeric pairs on a common HP-5 capillary (2, 3).

Supercritical fluid chromatography (SFC) also turned out to be a powerful alternative for the separation of new designer drugs. We published some results of this work with another research group (4).

Besides chromatography, we also developed chiral separation methods for capillary electrophoresis (CE) – a technique with a 25-year history in our lab. We tested several chiral selectors as additives to the background electrolyte. The use of cyclodextrin derivatives as well native ß-cyclodextrin was successful for various chiral compound classes, such as cathinones, amphetamines, benzofuries, thiophenes, phenidine, and phenidate derivatives. Furthermore, the use of a capillary packed with a cellulose-based chiral selector ended up with successful results; once again, this was done with another research group (5).

To date, we've been able to resolve the enantiomers of more than 100 of these compounds using HPLC (6, 7, 8) and CE (9, 10, 11). When compared with chiral GC-MS analysis, chiral separation with both techniques offers an additional benefit: we're able to differentiate between ortho- meta- and para-forms because of the different retention/migration times and chiral separation factors of the enantiomer pairs.

(Unexpected) NPS trends

In addition to the prominent chemical compound class of cathinones, native amphetamine and N-methamphetamine (both prohibited) also arrived on the scene with slight alterations; the first derivative showed up in 2011 as 4-fluoroamphetamine (4-FA), followed by 3-fluoroamphetamine (3-FA) and 2-fluoroamphetamine (2-FA). Later, 4-chloroamphetamine (4-CA) and 4-methylamphetamine (4-MA) were traded. Now, after methamphetamines, ethamphetamines are available.

The concept of introducing a beta-keto function to convert an amphetamine into a cathinone led to similar attempts in other classes. Ecstasy analogues are one prominent example: MDMA was replaced by MDMC (methylone). Other closely related substance classes also became very popular: benzofuries, tryptamines, and ketamines. Obviously, the growing number of new compounds reported to the European Monitoring Centre for Drugs and Drug Addiction each year was hard to keep track of (see Figure 2).

Over the years, I've found myself in a rather interdisciplinary network. Medical doctors ask about the name and structure of "bath salts" so that they can attempt to estimate their effects. Police and judges ask whether a certain new compound is scheduled or not. And I exchange the current "state of the art" with local forensic chemists periodically.

When the first compounds flooded Austria in 2010, I was sure that, eight years later, the majority of drug users would have switched to NPS instead of established drugs in a bid to avoid legal consequences. But, surprisingly, after the initial hype, we've seen a significant decline in official consumption of NPS – at least in Austria. The trend may be connected with the Austrian law on NPS, which came into force in 2012. As possession of NPS is allowed (and thus not prosecuted), it is difficult to estimate how many drug consumers actually joined the NPS revolution; despite a significant decrease in drug seizures by police, NPS use is still recorded at Austrian drug outpatient departments. In contrary, in Germany all NPS were added to the Betäubungsmittelgesetz – Narcotics Law – but, interestingly, a special law – Neue-psychoaktive-Stoffe-Gesetz (NpSG) also came into force in late 2016. Other countries, such as Switzerland or the UK, do not have special laws for NPS; instead, NPS are added to existing law (Single Convention 1961).

Given that any classic scheduled drug can be replaced by NPS nowadays (see Table 1), I'm surprised that they aren't more numerous.

More recently – in 2017 and 2018 – the four hot topics in the synthetic drug world have been: i) synthetic cannabinoids that

mimic delta-9-THC present in cannabis, ii) cathinones as the new bath salts, iii) new benzodiazepines – a totally new substance class to replace commonly available benzodiazepines, but without prescription, and, finally iv) dangerous fentanyl derivatives – dubbed "Opiates 2.0" – which have resulted in several fatalities.

The consumption of cannabis (or cannabinomimetics) is still the most popular form of substance (ab)use. Unsurprisingly then, cannabinomimetics are very popular right now – and are being produced from very different chemical substance classes. For consumers, these NPS offer several advantages: they are odorless crystals or liquids that can also be used with electronic devices such as e-cigarettes or e-shishas, and, compared with marijuana, much smaller amounts are sufficient to take effect. They are also more easily smuggled into prisons.

Another trend is the rapidly rising prevalence of cannabidiol-hemp, which is difficult to differentiate from marijuana. When hemp contains less than 0.3 percent delta-9-THC and belongs to the EU certified catalogue of brands, it is legal – but how can we quickly clarify this in the street? It seems to me that the cultivation of cannabidiol-hemp may be a targeted attempt to confuse authorities; after all, it is not so difficult to cultivate a small amount of drug-hemp hidden in a big plantation of cannabidiol-hemp…

The similarity between cannabidiol-hemp and drug-hemp also makes honest quality control problematic. To that end, a well-known vendor of HPLC-UV instrumentation now offers a specially adapted system – the "Cannabis Analyzer for Potency," which is able to determine several important cannabinoids in a single HPLC run.

Joining the good fight

GC-MS remains the most advantageous approach when it comes to the detection and identification of NPS. As most of the compounds are volatile, there is no need to undergo derivatization prior to analysis. Today, a database of designer drugs containing up to 26,500 mass spectra is commercially available.

My personal hope is for forensic analytical scientists to start working together much more closely – not only locally but also by building up an international network that can enable even more interdisciplinary collaboration. The goal for all scientists should be the same: to lend their clever minds and skills to the battle against the (perhaps hopeless) fight against drugs. NPS should never be regarded as "safe" – no matter what consumers think; after all, there is very little knowledge and few or no clinical studies in most cases. And clearly, given their novelty, there is no information regarding negative health effects from long-term exposure or dependence.

The abundance of emerging compound classes – despite the apparent official lull – is proof that there is no reason to give the all-clear on NPS just yet. Analytical chemists still have a vital role to play.

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Solutions

Real analytical problems
Collaborative expertise
Novel applications

Pharma QC Solution? SFC Stands Trial!

How an ambitious PhD project led to an even more ambitious world-first inter-laboratory study into super-critical fluid chromatography. The goal? To prove the technique's reproducibility in the most stringent of settings: pharmaceutical quality control.

By Amandine Dispas

When I started my PhD research work in 2016 under the supervision of Philippe Hubert at the University of Liège, Belgium, I found myself at the beginning of a renaissance in supercritical fluid chromatography (SFC). In 2011, SFC was still considered a niche technique – used only by several expert scientists. Phrases commonly appearing in the same sentence as SFC? High variability… poor robustness… technical issues (I remember conducting a few experiments with old equipment and it was quite a nightmare).

SFC certainly had advantages but, given the issues above, it was hard to imagine that pharmaceutical companies would one day use analytical SFC. But, thanks to the acetonitrile crisis in 2008, a need for greener technologies, and the provision of modern instrumentation, SFC was suddenly on the up! New instrumentation was presented as reliable, robust and compatible with sub 2 µm particles column. And now a much wider user base – in theory – could access the significant benefits of SFC: shorter analysis time, high efficiency, lower solvent use, and orthogonality. But what about in practice? Sounds like the perfect PhD project!

And so, the objective of my PhD was to

highlight the potential of analytical SFC for achiral pharmaceutical analysis. To that end, I demonstrated the quantitative performance of the technique by validating methods for several applications. Notably, in the last few years, there has been an increase in publications that demonstrate how SFC could easily replace normal phase liquid chromatography or ion-pairing LC with easier, cheaper and greener analysis. Nevertheless, SFC is not commonly accepted or used in the quality control laboratories.

I conducted the last part of my PhD project in collaboration with the University of Geneva (with Davy Guillarme) and Novartis R&D (with Adrian Clarke). Here, the objective was to develop an SFC method for the determination of pharmaceutical impurities as an alternative to the 'old fashioned' LC method found in the pharmacopoeias. We validated the method according to the pharmaceutical guidelines (ICH) and were amazed by the results; the project proved that SFC really could deliver optimal quantitation within a short analysis time.

Following on from this demonstration in one laboratory, we wanted to demonstrate that SFC still works in a highly demanding pharmaceutical domain. Thus, we ramped up the test to the inter-laboratory level to evaluate method reproducibility. The beginning of an exciting and enjoyable collaborative project.

A clear objective, a clear plan

During the validation step, you evaluate two levels of method precision: the repeatability and the intermediate precision. To evaluate the third level – the reproducibility – you have to add the "laboratory" variable by performing an inter-laboratory study. This entire topic can cause confusion because, in the literature, reproducibility terminology is often wrongly used. Nevertheless, the (proper) evaluation of reproducibility is mandatory when demonstrating that a new method is a suitable replacement for the reference method within the pharmacopeia. And an inter-laboratory study helps calculate method variance considering all sources of variability. Can method variance be at least similar to a reference technique (LC)? A question with a big potential impact: the answer could be considered as the "go/no go" sign for SFC in pharmaceutical quality control.

We planned this project during the second part of 2016. The first step was to define the study protocol, and also to write a standard operating procedure for the participating labs. Indeed, each analytical scientist has to follow exactly the same protocol (including SFC parameters, samples and standards preparation, injection sequence, and so on) to get reliable data. Furthermore, we also imagined a preliminary test to verify that each lab could perform the quantitative study. I put a great deal of effort into the

Living in a material world

Before contacting the potential participating labs, we needed to ensure that we could provide all the materials necessary for the project! Each lab around the world needed the same column, all the certified standards from the pharmacopeia, the samples and a detailed protocol – combined it represented around 2000 euros for each lab. (It's a common problem: you have an amazing research project in mind but you always need the funding to realize them!) Thankfully, Waters provided one DEA column to each lab, while Novartis provided the funding to get the certified standards to all laboratories.

But funding was just the first problem solved. Another big (and ongoing) challenge was coordinating a project that involved 19 research teams from universities, pharmaceutical companies, and demo labs all around the world. For one thing, your samples will be subject to different import/export and customs rules (we wanted to work with one lab in China, but it was impossible to send the samples). After sending the parcels with samples and standards to each lab, I found myself checking delivery tracking several times per day.

Clearly, we wanted to work with reliable data but, when it comes to SFC, equipment differences in dead volume, supercritical fluid pumping, pressure regulation, UV cell geometry, and so on can all have an impact on the method selectivity and efficiency. And that's why we chose to work with a single type of instrument: the UPC2 (Waters). We also asked each operator to work only with UV-PDA and remove other detector(s) connections (for example, MS, ELSD) to further standardize instrument configurations.

study protocol, report template and excel sheets for results calculation, because these aspects were so critical for the credibility of the study. I was very grateful to Davy and Adrian, who both reviewed the protocol. And several tests were done both in my lab and in Davy's lab to validate the protocol, the report template and the excel sheets. The work seemed almost never ending at the time, but such planning is a keystone for such studies.

In the beginning of 2017, we selected the potential participants – a task made easier through Davy and Adrian's SFC contacts! We thought it was important to include scientists from different domains: universities, demo labs and pharmaceutical companies. And we ended up with 16 potential participants (in addition to our three labs). We contacted them to explain our project and asked if they would like to participate. Rather incredibly (at least to me), we quickly received enthusiastic and positive answers from everyone. It felt like we'd brought together a community of scientists who were already convinced by SFC's potential and so wanted to promote this technology. Furthermore, the scientists in pharmaceutical companies, seemed like they wanted to prove to management that SFC is able to fulfil the regulatory requirements.

The laboratories received column, samples, standards at the beginning of June 2017. The first part of the experiments was a "system suitability test" (SST) to evaluate the performance of each lab regarding the separation of all compounds, the system repeatability and sensitivity. I wanted to be sure that each SFC instrument worked properly and each lab could reach the separation before performing the quantitative study. I received all SST results and gave the thumbs for samples analysis! I got all reports at the end of September. In the meantime, I checked sample stability each month to validate the analysis time window in all labs (from June to September).

The big reveal

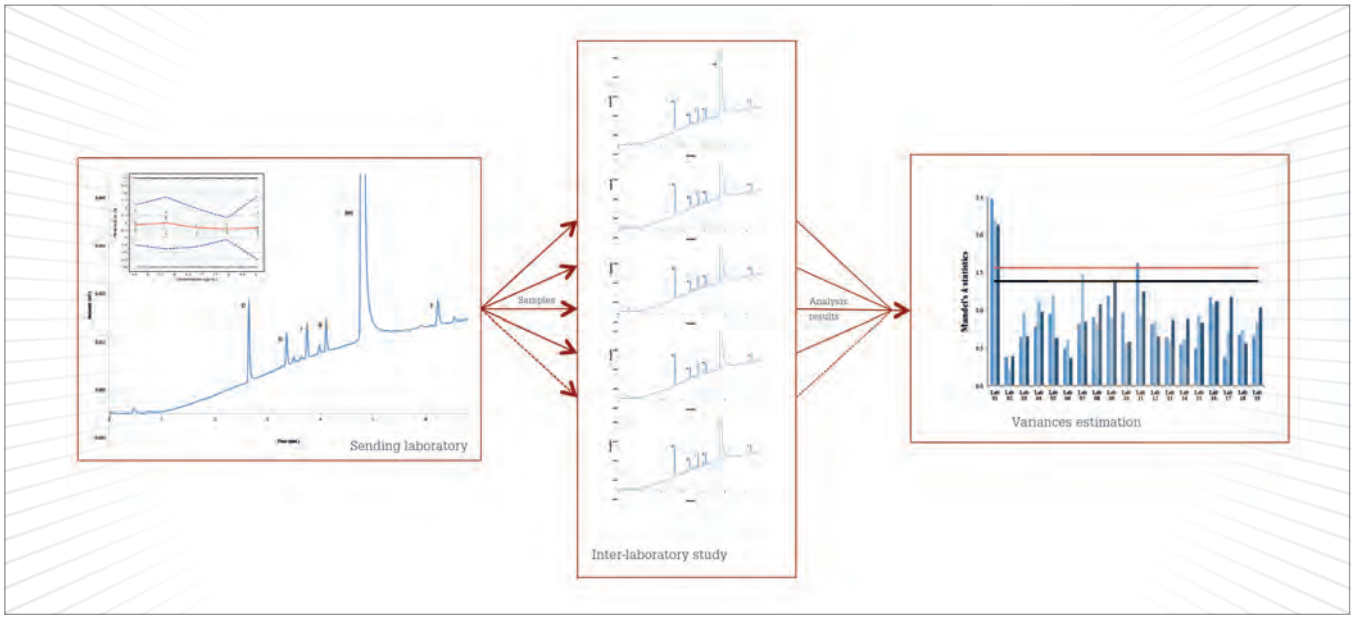
Statistical data analysis was the final – and admittedly tedious – part of the project. There was a huge amount of data (19 laboratories, three samples per laboratory, three series of analyses per sample, three replicates per series). Fortunately, ISO guidelines provide several tools to assess method precision, which helped with the organization of my workload. Firstly, I checked the data with regards to consistency and found one laboratory to be an outlier. So thanks to this first report, we'd identified the root cause of outlier results and were able to discard this single lab for the evaluation of method variance. Secondly, I estimated the variance considering all sources of variability (which is to say replicates, series, laboratories).

We finalized our statistical analyses at the end of 2017 and sent them to a chemometrics expert – Yvan Vander Heyden (Vrije Universiteit Brussel) – for review.

Inter-laboratory reproducibility was similar or better than that reported in the literature for the reference technique (1,2). I could finally say: "Yes! It works!" For several years, we've been working to highlight the performance of SFC for QC analysis, and now we have the ultimate demonstration. Scientifically, it is the first published study demonstrating that SFC suits pharmaceutical requirements. And, in my opinion, that makes this project a real success.

Davy Guillarme presented the work at SFC 2018 in Strasbourg, France, and

Exhibit A. Simplified outline of "the trial."



everyone really enjoyed hearing about the project. We also had a lot of stimulating discussions with SFC users.

As for industrial impact? I don't think SFC will replace LC as the reference technique just because of our study! However, I do feel that perceptions of SFC have change, especially for people working in pharma companies. They now know that the technique fulfils all the pharmaceutical requirements. In the future, we can imagine that SFC will be implemented for new products or when modifying old fashioned methods.

One pharma scientist told me that he showed our publications to the management board of his pharma company to help him move forward with SFC. And they just submitted their first marketing authorization involving SFC as QC method – a wonderful anecdote! I guess this study could be considered the first page of a new life for SFC.

Not done yet…

We planned this study using one type of SFC instrument (Waters UPC2). Obviously, we want to extend the approach to other instrumentation used in the QC labs. SFC method transfer is not as easy as LC, you have to manage the

Super Team

Davy Guillarme, University of Geneva
This project represents the first inter-laboratory study involving SFC (given the total cost, perhaps that's not surprising!). To ensure good statistics – and to allow exclusion of possible outlier laboratories – a large number of laboratories need to be involved in a any valid inter-laboratory. But I think one particular strength of this work was that academic laboratories, industrial laboratories, and vendor demo labs were all included; despite potentially strong differences in terms of practice, the results were still very good.

One unexpectedly challenging aspect was that it was hard to suggest potential reviewers for the paper we submitted – almost all of the main SFC players were involved in this inter-laboratory study!

To conclude, I would say that this study represents a major step forward for SFC in quality control (QC) laboratories, particularly in the pharmaceutical industry. The work proves that SFC can be included within the arsenal of methods used for QC of drugs, alongside HPLC and GC.

Caroline West, University of Orléans
I agree with Davy that, although having a large number of laboratories involved in the project was certainly important, having laboratories with very different constraints and practices (in terms of maintenance, quality management system) was even more significant. It ought to be even more convincing to SFC-skeptics that, with such a diversity of laboratories involved, the results were very good in the end.

SFC has long had a reputation of lacking robustness. And though I was never overly concerned because I found that its significant benefits outweighed any negatives, I know that many people were deterred from trying it because of a (now wholly undeserved) bad reputation. I hope this very important milestone in SFC evolution will help the skeptics finally jump in!

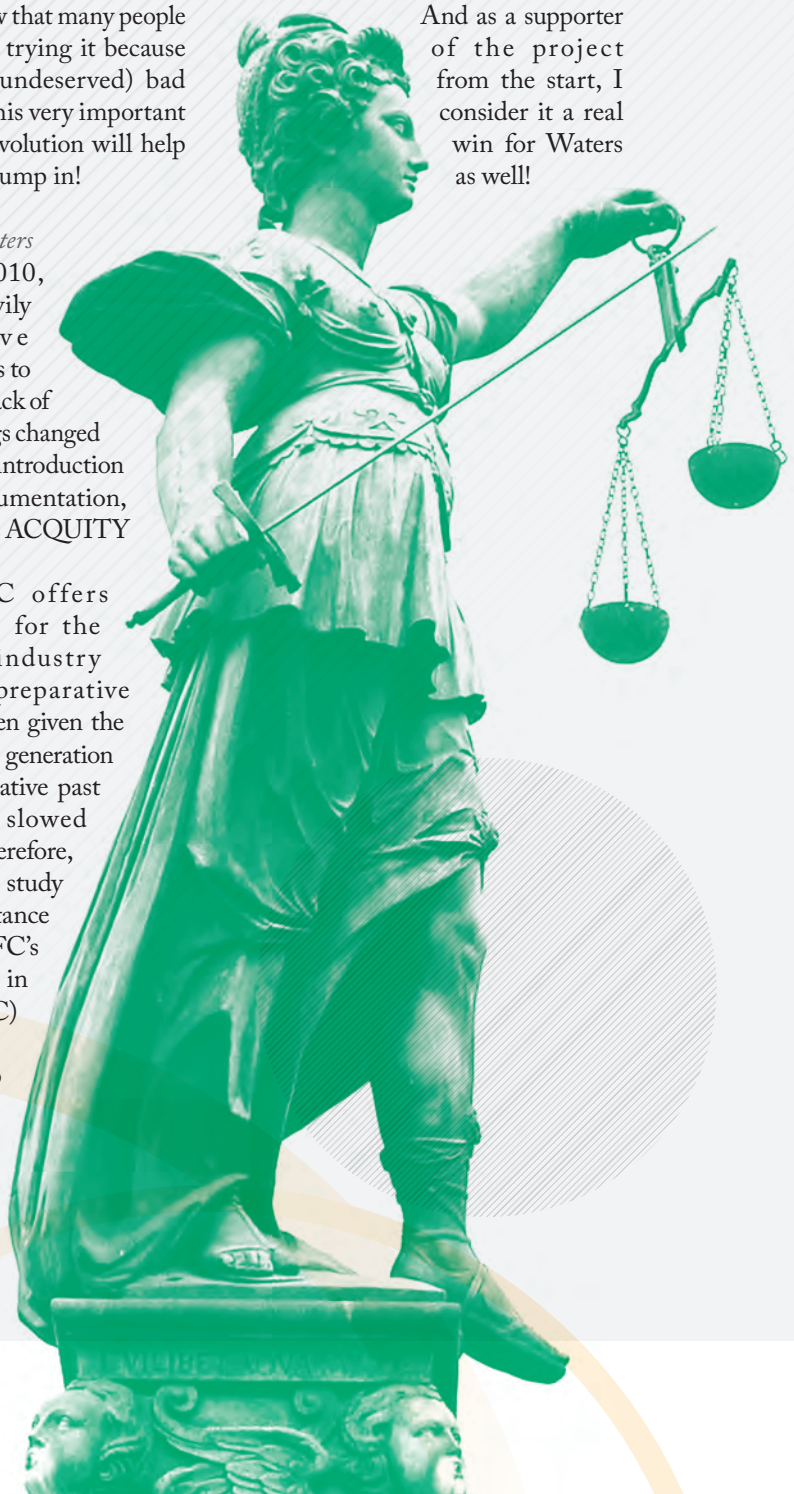
Isabelle François, Waters
Until the year 2010, SFC struggled heavily with a negative perception in regards to reproducibility and lack of UV sensitivity. Things changed drastically with the introduction of new SFC instrumentation, including the Waters ACQUITY UPC^2, in 2012.

Although SFC offers many advantages for the pharmaceutical industry (and not just in preparative applications) and even given the availability of a new generation of instruments, negative past experiences have slowed down adoption. Therefore, this inter-laboratory study is of crucial importance in demonstrating SFC's applicability – even in quality control (QC) environments.

The study will help early adopters get more recognition for the technology internally, so that they may promote more widespread use across their organizations.

From a scientific standpoint, the study has been a great success – and serves as a wonderful promotion for SFC technology in general.

And as a supporter of the project from the start, I consider it a real win for Waters as well!



supercritical fluid, regulate the pressure, and so on. Moreover, each instrument has a specific way of injecting a liquid into the supercritical mobile phase. Now, we are working with Agilent and Shimadzu to test their systems by properly transferring method before starting a new study in 2019.

I have other interesting – but confidential – SFC projects with two PhD students, and I'm delighted to see new researchers wanting to push SFC to the limit. I've also been working in the laboratory of Marianne Fillet (University of Liège) on the miniaturization of a CE instrument... Another testing but enjoyable challenge!

Going back to the study at the heart of this article – and my time in SFC in general – I feel that I've been blessed with great scientific networking throughout. We've worked closely with people all around the world on a unique and common objective – and that's made it an amazing experience.

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Amandine Dispas is a Postdoctoral Research Scientist at the Laboratory of Pharmaceutical Analytical Chemistry & Laboratory for the Analysis of Medicines, Univeristy of Liège, Belgium.

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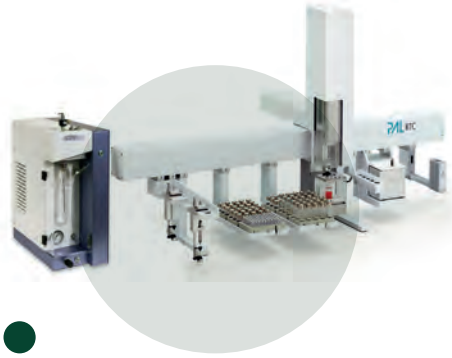

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PHARMA ANALYSIS: THE NEXT GENERATION

Sitting Down With…
Jean-Luc Veuthey,
Professor, School of Pharmaceutical Sciences, University of Geneva, Switzerland.

What does a typical day look like for you?
My working day is divided between administrative tasks, research activities (discussing ideas with my PhD students and colleagues, writing articles and so on) and teaching activities (preparing for and delivering lectures, marking essays, and so on). Of these activities, it is the interaction with students and colleagues that I find most motivating – it was teaching that I missed most when I spent a few years in industry. The best moments for me are the PhD defenses of my students – it's wonderful to see their efforts come to fruition. Over the next few years I plan to expand on my work in education, by developing new teaching activities for the next generation of students, both here and in developing nations.

What makes the University of Geneva a good place to do research?
I was born in Geneva and I believe it is a special city – it is a melting pot of different languages and cultures, giving it an open-minded outlook. The University has a strong focus on collaboration, both within Switzerland, Europe and internationally – including developing countries. I am lucky to have a lot of freedom to follow my interests and choose collaborators to develop innovative projects.

What is the core focus of your research?
It is important to me to focus my research on applications that have major societal impacts, including investigating drugs of abuse, medicine for our aging population, sports doping, environmental impacts and new therapeutic drugs. For the last 10 years, my group and I have developed more rapid and efficient separation methods (including sample preparation) for the analysis of small drugs in biological matrices and biopharmaceuticals in drug formulation. We also carry out fundamental studies in separation sciences to help improve the tools available for these important applications. In future, I'm interested in looking at cannabis testing – its an area where legislation is changing fast, and it's important we understand what problems might result.

Such a strong focus on applications must require solid collaboration…
Finding the right partners is critical to our work. The lab is well integrated in the School of Pharmaceutical Sciences and we have several collaborations with colleagues at the University involved in pharmaceutical technology, medicinal chemistry, phytochemistry and clinical pharmacy – both in Geneva and Lausanne. The lab also has strong links with colleagues in the Faculty of Medicine and with a number of private and public external laboratories. The lab is also member of The Center of Competence in Analytical Chemistry and Toxicology (ccCTA), which includes several laboratories in western Switzerland.

What is your teaching style?
A significant part of the education we provide is practice-oriented, with 50 percent of the week spent in the lab. Master's students must also organize two internships (20–25 weeks) in community pharmacies and research laboratories in industry or university.

When it comes to lectures, I believe that the way we teach needs to move with the times. Today's students have constant access to technology, and that has an impact on their attention span. For some of my lectures I am completely changing the traditional format. I have prepared 5-min videos explaining some challenging topics and ask students to watch them before the lecture. I then cut my lecture down to 15-20 mins and spend the rest of the time working with students on group exercises and giving immediate feedback. I also use anonymous online tests to regularly check students' understanding, and adapt teaching sessions to focus on questions they struggled with. These modern teaching methods have improved exam scores – and both lecturers and students enjoy the increased interaction.

"We have seen amazing advances in telecommunications – why can't we see the same leaps in technology in separation science?"

How do you contribute to ongoing innovation in the analytical sciences?
We develop new separation strategies and share our knowledge with instrument and chromatographic column providers. For specific applications, we also modify the instrumentation and patent the results. In Switzerland, there is a strong support from the Swiss National Science Foundation and others to conduct fundamental studies and patent innovative new tools.

What does the future hold for pharmaceutical analysis?
I would like to see the development of simple and robust analytical tools at a lower price than those currently available. These tools could be used for point-of-care testing by non-specialists, for example doctors, pharmacists or patients. In recent years, we have seen amazing advances in telecommunications devices – why can't we see the same leaps in technology in separation science? I am always impressed by the activities of George Whitesides' research group in this domain.

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